# PHASE II WINDOW OF OPPORTUNITY TRIAL OF STEREOTACTIC BODY RADIATION THERAPY AND IN SITU GENE THERAPY FOLLOWED BY NIVOLUMAB IN METASTATIC SQUAMOUS OR NON-SQUAMOUS NON-SMALL CELL LUNG CARCINOMA AND METASTATIC UVEAL MELANOMA.

## ENSIGN TRIAL


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## List of abbreviations

- ADV: Adenovirus-mediated
- AE: Adverse event
- ALT: Alanine aminotransferase
- ANC: Absolute neutrophil count
- AST: Aspartate transaminase
- AUC: Area under the curve
- β-hCG: β-human chorionic gonadotropin
- BUN: Blood urea nitrogen
- CBC: Complete blood count
- CBR: Clinical benefit rate
- CI: confidence interval
- Cmax: Maximum plasma concentration
- CR: Complete response
- CRF: Case report form
- CRT: Calreticulin
- CT: Computed tomography
- CTCAE: Common Terminology Criteria for Adverse Events
- CTL: Cytotoxic T lymphocyte
- CTLA-4: Cytotoxic T-lymphocyte-associated antigen-4
- CXCL16: Chemokine ligand 16
- DAMP: Danger-associated molecular pattern
- DC: Dendritic cell
- DoR: Duration of response
- DSMP: Data and safety monitoring plan
- ECG: electrocardiogram
- ECHO: echocardiogram
- ECOG: Eastern Cooperative Oncology Group
- EOT: End of treatment
- EUS: Endoscopic ultrasound
- FDA: Food and Drug Administration
- GCV: Ganciclovir
- GM-CSF: Granulocyte macrophage colony-stimulating factor
- Gy: Gray
- HD: High-dose
- HIV: Human immunodeficiency virus
- HMGB1: High-mobility group protein 1
- HMRI: Houston Methodist Research Institute
- HSP: Heat shock protein
- HSV: Herpes simplex virus
- HUS: Hemolytic uremic syndrome
- IFN: Interferon
- Ig: Immunoglobulin
- IL: Interleukin

- IND: Investigational new drug
- IRB: Institutional review board
- IRC: Independent review committee
- irRC: Immune-related response criteria
- IUD: Intrauterine device
- IUS: Intrauterine system
- IV: intravenous
- MHC: Major histocompatibility complex
- mDC: Monocyte-derived DC
- MUGA: Multigated acquisition
- NCI: National Cancer Institute
- NK: Natural killer
- NKG2D: Natural killer group 2, member D
- NSCLC: Non-small cell lung carcinoma
- ORR: Objective response rate
- OS: Overall survival
- PAMPs: Pathogen-associated molecular patterns
- PD: Progressive disease
- PR: Partial response
- PD-1: Programmed death 1
- PD-L1: Programmed death-ligand 1
- PDL-2: Programmed death-ligand 2
- PET: Positron emission tomography
- PFS: Progression-free survival
- PSA: Prostate specific antigen
- QD: Daily
- RAC: Regulatory Affairs Certification
- RECIST: Response Evaluation Criteria in Solid Tumors
- RT: Radiation therapy
- SAE: Serious adverse event
- SBRT: Stereotactic body radiation therapy
- SD: Stable disease
- Th1: T-helper type-1
- t.i.d.: ter in die; three times daily
- TIL: Tumor infiltrating lymphocyte
- tk: Thymidine kinase
- TLR: Toll-like receptor
- TNBC: Triple negative breast cancer
- TNF-α: Tumor necrosis factor-α
- Treg: Regulatory T cell
- TTP: Thrombotic thrombocytopenic purpura
- ULN: Upper limit of normal
- VZV: Varicella-zoster virus
- WBC: White blood cell
- WOCBP: Women of childbearing potential

## Glossary of terms

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| Assessment | A procedure used to generate data required by the study |
| Baseline | For efficacy evaluations, the baseline assessment will be the last available assessment before or on the date of randomization.<br>For safety evaluations (i.e. laboratory assessments and vital signs), the baseline assessment will be the last available assessment before or on the start date of study treatment.<br>The value obtained at baseline assessments, referred to as "baseline value" will be used as reference for the patient. |
| Control drug | A study treatment used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug |
| Dose level | The dose of drug given to the patient (total daily or weekly etc.) |
| Enrollment | Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol) |
| Investigational drug | The study treatment whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug." |
| Investigational treatment | Drug whose properties are being tested in the study as well as their associated placebo and active treatment controls (when applicable). This also includes approved drugs used outside of their indication/approved dosage, or that are tested in a fixed combination. Investigational treatment generally does not include other study treatments administered as concomitant background therapy required or allowed by the protocol when used in within approved indication/dosage |
| Medication number | A unique identifier on the label of each study treatment package which is linked to one of the treatment groups of a study |
| Other study treatment | Any drug administered to the patient as part of the required study procedures that was not included in the investigational treatment |
| Patient | A unique identifying number assigned to each patient/subject/healthy volunteer who enrolls in the study |
| Period | A subdivision of the study timeline; divides stages into smaller functional segments such as screening, baseline, titration, washout, etc. |
| Premature patient withdrawal | Point/time when the patient exits from the study prior to the planned completion of all study treatment administration and/or assessments; at this time all study treatment administration is discontinued and no further assessments are planned, unless the patient will be followed for progression and/or survival |
| Stop study participation | Point/time at which the patient came in for a final evaluation visit or when study treatment was discontinued whichever is later |
| Study treatment | Includes any drug or combination of drugs in any study arm administered to the patient (subject) as part of the required study procedures, including placebo and active drug run-ins.<br>In specific examples, it is important to judge investigational treatment component relationship relative to a study treatment combination; study treatment in this case refers to the investigational and non-investigational treatments in combination. |
| Study treatment discontinuation | Point/time when patient permanently stops taking study treatment for any reason; may or may not also be the point/time of premature patient withdrawal |
| Supportive treatment | Refers to any treatment required by the exposure to a study treatment, e.g. premedication of vitamin supplementation and corticosteroid for pemetrexed disodium. |
| Variable | Identifier used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified time points |

## 1. Background

### 1.1 Overview of disease pathogenesis, epidemiology, and current treatment

Lung cancer is the leading cause of cancer death in the United States. Lung and bronchial cancer are expected to account for an estimated 221,200 new cases (115,610 in men and 105,590 in women) and 158,040 deaths (86,630 in men and 71,660 in women) in 2015.[1] Only 16.8% of lung cancer patients are alive for 5 years or more after their diagnosis. Non-small cell lung carcinoma (NSCLC) accounts for over 85% of all lung cancer cases. Most patients are diagnosed with advanced or metastatic (stage IIIB/IV) disease, [2] and current first-line treatment options for these patients are limited. In patients with advanced NSCLC, first-line platinum-based doublet chemotherapy yields 1-year overall survival (OS) rates of only 30–40% and can cause significant toxicities that may complicate treatment.[2] However, much progress has recently been made in the diagnosis and treatment of lung cancer including screening methods, minimally invasive techniques for diagnosis and treatment, and advances in radiation therapy (RT), including stereotactic body RT (SBRT), targeted therapies, and immunotherapies.[3-6]

Uveal melanoma represents approximately 85% of all ocular melanomas. Metastatic disease develops in up to 50% of patients and is associated with poor prognosis.[7] The most frequent site of metastasis is the liver (95%), followed by the lungs (24%), bone (16%), and skin (11%).[8,9] Median survival for patients with liver metastases is approximately 4−6 months with a 1-year survival of approximately 10−15%, whereas patients with metastases not involving the liver have a median survival of approximately 19−28 months with a 1-year survival of approximately 76%.[10-12] There is no proven treatment for metastatic uveal melanoma, and standard therapies for cutaneous melanoma are often adopted but show limited activity.[11] As uveal and cutaneous melanomas exhibit marked differences in their molecular features,[13] dedicated therapies for metastatic uveal melanoma are needed to improve the outcomes of patients with this hard-to-treat disease.

Cancer immunotherapy, which harnesses and boosts the innate powers of the immune system to fight cancer, represents the most promising new cancer treatment approach since the development of chemotherapeutic agents in the late 1940s. Because of the extraordinary memory and specificity of the immune system, immunotherapy has the potential to achieve complete, long-lasting remissions with few or no side effects in cancer patients, regardless of their cancer type. Therapies that target programmed death 1 (PD-1) have shown unprecedented rates of durable clinical responses in patients with various cancer types.[14-18] One mechanism by which cancer tissues limit the host immune response is via upregulation of programmed death-ligand 1 (PD-L1) and its ligation to PD-1 on antigen-specific $CD8^+$ T cells (termed adaptive immune resistance).[19, 20]

The number of immunotherapy clinical trials has dramatically increased in the past decade. These trials have shown the promise of immunotherapy in enhancing antitumor immune responses. Several clinical studies have expanded the list of cancers that can be treated with checkpoint blockade therapy. Brahmer et al. demonstrated that, among patients with previously treated advanced squamous-cell NSCLC, OS, response, and progression-free survival (PFS) rates were significantly better with the PD-1 inhibitor nivolumab than with docetaxel regardless of PD-L1 expression level.[21] The confirmed objective response rate (ORR) was significantly higher with nivolumab than with docetaxel (20% [95% confidence interval [CI]: 14–28] vs. 9% [95% CI: 5–15]; P = 0.008)[21]. Herbst et al. found that treatment with MPDL3280A, a PD-L1-specific monoclonal antibody, induces therapeutic responses in patients with advanced NSCLC, renal cell cancer, and melanoma.[22] Powles et al. showed that the same antibody can be used to treat urothelial bladder cancer.[23] Among patients with a minimum of 6 weeks of follow-up, ORRs were 43% (13/30; 95% CI: 26–63) for those with tumor PD-L1 immunohistochemical expression scores of 2 or 3 (2/3) and 11% (4/35; 95% CI: 4–26) for those with tumor PD-L1 immunohistochemical expression scores of 0 or 1 (0/1). In the tumor PD-L1 immunohistochemical expression 2/3 score group, the ORR included a 7% complete response rate (2/30). Among patients with a tumor PD-L1 immunohistochemical expression score of 2/3 and a minimum of 12 weeks of follow-up, an ORR of 52% (13/25; 95% CI: 32–70) was achieved. Sixteen of the 17 responders had ongoing responses, and all 17 responders continued on MPDL3280A treatment until the data cutoff date. One patient who initially responded at the first response assessment later presented with new lesions,

including a bladder mass thought to be consistent with pseudo-progression.[23] Anti-PD-L1 immunotherapy has also shown promising results in triple negative breast cancer (TNBC).[24] The latest data analysis of a phase I clinical trial of MPDL3280A in TNBC revealed a 24-week PFS rate of 27% and ORR rate of 19%, with three of four responses ongoing. This data is encouraging, because longer responses do not typically occur in metastatic TNBC patients treated with chemotherapy, which is the standard of care for this population.[17] Together, these cancer immunotherapy studies have reported durable responses and low toxicity rates. This is particularly important as high-grade adverse effects have limited the use of immunotherapy for cancer treatment in the past. PD-L1/PD-1 expression in cancer cells is an obvious candidate biomarker for immunotherapy response, as PD-L1/PD-1 can directly turn off the immune response by inhibiting the activity of tumor-infiltrating cytotoxic T lymphocytes (CTLs). However, PD-L1 and PD-1 expression in tumor cells has been shown to have little predictive power. On the other hand, Herbst et al. reported that PD-L1 expression in immune cells is a good biomarker of response to immunotherapy.[15] The finding that the complexity of the T cell population in the tumor infiltrate can predict good response to checkpoint blockade therapy highlights the importance of identifying tumor antigens that can elicit an effective antitumor immune response. Previous studies have suggested that tumors with a high load of somatic mutations are more likely to respond to immunotherapy, as in theory these tumors would have a higher diversity of neoantigens that can trigger an immune response when CTL-lymphocyte-associated antigen-4 (CTLA-4)/PD-1 inhibition is bypassed.

**Nivolumab BMS-936558**, marketed as Opdivo, is a fully human immunoglobulin (Ig) G4 anti-PD-1 monoclonal antibody developed by Bristol-Myers Squibb for the treatment of cancer.[25] Nivolumab acts as an immunomodulator by blocking PD-L1 binding to PD-1 on activated T cells. PD-1 is a protein expressed on the surface of activated T cells. The binding of PD-L1 or PD-L2 to PD-1 results in T cell inactivation. PD-L1/2-mediated T cell inactivation acts as an immune regulatory mechanism to avoid immune overreaction. Many cancer cells make PD-L1, allowing them to disarm T cells and inhibit their attack on tumor cells. Nivolumab blocks PD-L1 from binding to PD-1, allowing T cells to attack the tumor. PD-1 blockers appear to free up the immune system only around the tumor, rather than more generally, which may result in fewer side effects.[26] A phase 1 dose optimization trial of nivolumab was performed in patients with melanoma, lung cancer, kidney cancer, and other cancers. Among the 107 melanoma patients in this trial, nivolumab demonstrated 1-, 2-, and 3-year survival rates of 62%, 48%, and 41%, respectively. Toxicities, which were not cumulative and mostly occurred during the first 6 months of therapy, included pneumonitis, low-grade fatigue, diarrhea, pruritus, nausea, and decreased appetite. Pneumonitis was the most important adverse effect, leading to 3 deaths. Twenty-two percent of patients in the trial experienced a treatment-related grade 3 or 4 toxicity.[27, 28] A second phase 1 trial examined nivolumab in combination with ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with metastatic melanoma. Among 53 patients treated concurrently with the highest dose (1 mg/kg nivolumab and 3 mg/kg ipilimumab), 53% had an objective response; these patients all had a reduction in tumor volume of 80% or more. An updated report from this trial, including an additional 41 people treated with the highest dose, stated that 79% of patients were still alive after 2 years.[29] In a phase 3 trial, nivolumab monotherapy was found to be superior to traditional chemotherapy in terms of response rate (40% vs. 13.9%), PFS (5.1 months vs. 2.2 months), and percentage of patients still alive after 1 year (72.1% vs. 42.1%). Nivolumab received Food and Drug Administration (FDA) approval for the treatment of melanoma in December 2014.[30]

Nivolumab has also demonstrated efficacy in lung cancer cohorts. A phase 1b trial of nivolumab was conducted in patients with various cancers, including 129 patients with NSCLC.[29] Most NSCLC patients had received multiple chemotherapies. The overall response rate in this group was 17%, with a median duration of response (DoR) of 74 weeks. At the highest dose, 45% of patients were still alive after 2 years.[29] A multiarm phase 1 trial examined the efficacy of nivolumab as a single agent or as part of combination therapy.[28] In the first arm, 56 chemotherapy-naïve patients were treated with nivolumab plus cisplatin/gemcitabine, cisplatin/pemetrexed, or carboplatin/paclitaxel. Response rates ranged from 33–50%, and 59–87% of the patients were still alive after 1 year. Serious adverse events (SAEs) occurred in 45% of patients. In a second arm, chemotherapy-naïve patients were treated with nivolumab monotherapy. Of these 20 patients, 71–80% were still alive after 1 year. In a third

arm, patients were treated with a combination of nivolumab and ipilimumab. Median OS in this group was 44.3 weeks. In a phase II safety trial of nivolumab (3 mg/kg ever 2 weeks) for patients with refractory metastatic NSCLC, 20 (17%) of 117 patients reported grade 3-4 treatment-related adverse events (AEs), including fatigue (5/117 [4%]), pneumonitis (4/117 [3%]), and diarrhea (3/117 [3%]). Two treatment-associated deaths caused by pneumonia and ischemic stroke occurred in patients with multiple comorbidities in the setting of progressive disease. In the phase 3 CheckMate-017 trial, nivolumab improved survival compared with docetaxel in previously treated patients.[31, 32]

Nivolumab has demonstrated efficacy in the treatment of metastatic squamous and non-squamous NSCLC. In the phase III CheckMate-017 trial, 272 patients with metastatic squamous NSCLC who had progressed during or after one prior platinum doublet-based chemotherapy regimen were randomized (1:1) to receive nivolumab (n=135; 3 mg/kg intravenous [IV] every 2 weeks) or docetaxel (n=137; 75 mg/m$^2$ IV every 3 weeks) in an open label study.[33] This study included patients regardless of their PD-1 status. Patients with autoimmune disease, symptomatic interstitial lung disease, or untreated brain metastasis were excluded. Patients with treated brain metastases were eligible if their neurological status had returned to baseline at least 2 weeks prior to enrollment and they were either off corticosteroids or on a stable or decreasing dose of <10 mg daily prednisone equivalents. Tumor assessments were conducted 9 weeks after randomization and every 6 weeks thereafter. The major efficacy outcome measure was OS. The median OS was 9.2 months (range: 7.3–13.3 months) and 6.0 months (range: 5.1–7.3 months) for nivolumab and docetaxel, respectively (P = 0.00025). The median patient age was 63 years (range: 39–85 years); 44% and 11% of patients were ≥ 65 and ≥ 75 years of age, respectively. The majority of patients were white (93%) and male (76%). Baseline Eastern Cooperative Oncology Group (ECOG) performance status was 0 in 24% of patients and 1 in 76% of patients. This trial demonstrated a statistically significant improvement in OS with nivolumab as compared with docetaxel at the prespecified interim analysis conducted when 199 events were observed (86% of the planned number of events for the final analysis). A single-arm, multinational, multicenter trial of nivolumab was conducted in patients with metastatic squamous NSCLC whose disease had progressed after receiving a platinum-based therapy and at least one additional systemic treatment regimen. [26] This study included patients regardless of their PD-1 status. Nivolumab (3 mg/kg) was administered intravenously over 60 minutes every 2 weeks. Patients with autoimmune disease, symptomatic interstitial lung disease, or untreated brain metastasis were excluded. Patients with treated brain metastases were eligible if their neurological status had returned to baseline at least 2 weeks prior to enrollment and they were either off corticosteroids or on a stable or decreasing dose of <10 mg daily prednisone equivalents. Tumor assessments were conducted 8 weeks after treatment start and every 6 weeks thereafter. The major efficacy outcome measure was confirmed ORR as measured by an independent review committee (IRC) using the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). Additional outcome measures included DoR. A total of 117 patients received treatment with nivolumab. The median age was 65 years (range: 37–87 years); 50% of patients were ≥ 65 years of age and 14% of patients were ≥ 75 years of age. The majority of patients were male (73%) and white (85%). All patients received two or more prior systemic treatments; 35% received two, 44% received three, and 21% received four or more. At baseline, 6%, 94%, and 1.7% of patients had recurrent stage IIIb disease, stage IV disease, and brain metastases, respectively. Baseline ECOG performance status was 0 in 22% of patients and 1 in 78% of patients. Based on IRC review and with a minimum follow-up of at least 10 months on all patients, the confirmed ORR was 15% (17/117; 95% CI: 9–22), of which all were partial responses. The median time to onset of response was 3.3 months (range: 1.7–8.8 months). Thirteen of the 17 patients (76%) with a confirmed response had ongoing responses with durations ranging from 1.9+ to 11.5+ months; 10 of these 17 (59%) patients had durable responses of 6 months or longer.

In the open-label, phase III CheckMate-057 trial, 582 patients with metastatic non-squamous NSCLC who had experienced disease progression during or after one prior platinum doublet-based chemotherapy regimen were randomized (1:1) to receive second-line nivolumab (n=292) or docetaxel (n=290).[34] Appropriate prior targeted therapy in patients with known sensitizing *epidermal growth factor receptor* (*EGFR*) mutation or *anaplastic lymphoma kinase* (*ALK*) translocation was allowed. Nivolumab (3 mg/kg) and docetaxel (75 mg/m$^2$) were administered intravenously every 2 and 3 weeks, respectively. Randomization was stratified by prior maintenance

therapy (yes vs. no) and number of prior therapies (1 vs. 2). The trial excluded patients with autoimmune disease, medical conditions requiring systemic immunosuppression, symptomatic interstitial lung disease, or untreated brain metastasis. Patients with treated brain metastases were eligible if neurologically stable. The first tumor assessments were conducted 9 weeks after randomization and every 6 weeks thereafter. The median patient age was 62 years (range: 21–85 years), with 42% of patients ≥ 65 years of age and 7% of patients ≥ 75 years of age. The majority of patients were white (92%), male (55%), and former/current smokers (79%). Most patients were enrolled in Europe (46%), followed by the US/Canada (37%) and the rest of the world (17%). Baseline ECOG performance status was 0 in 31% of patients and 1 in 69% of patients. *ALK* rearrangement and *EGFR* mutation were present in 3.6% and 14% of patients, respectively, and 12% of patients had previously treated brain metastases. Prior therapy included platinum-doublet regimen (100%), and 40% of patients had received maintenance therapy as part of their first-line regimen. Histologic subtypes included adenocarcinoma (93%), large cell (2.4%), and bronchoalveolar (0.9%). The major efficacy outcome measure was OS. Nivolumab significantly improved median OS compared with docetaxel at the prespecified interim analysis conducted when 413 events were observed (93% of the planned number of events for final analysis; 12.2 months vs. 9.4 months; P = 0.0015). The median DoR was 17 and 6 months with nivolumab and docetaxel, respectively. Complete responses (CRs) and partial responses (PRs) were achieved in 4 (1.4%) and 52 (18%) patients in the nivolumab arm and 1 (0.3%) patient and 35 (12%) patients in the docetaxel arm. PFS was 2.3 and 4.2 months in the nivolumab and docetaxel arms, respectively. Based on the results of these trials, nivolumab has been approved for the treatment of metastatic squamous and non-squamous NSCLC with progression on or after platinum-based chemotherapy. Patients with *EGFR* mutation or *ALK* translocation should have disease progression on appropriate targeted therapy prior to receiving nivolumab.

Nivolumab has demonstrated significant single-agent activity in metastatic melanoma. Results from CheckMate 066 (NCT01721772)[35] and CheckMate 067 (NCT01844505)[36] led to the approval of nivolumab for the first-line treatment of BRAF V600 wild-type and mutation-positive unresectable or metastatic melanoma. The majority of subjects in these trials had cutaneous melanoma; subjects with uveal melanoma were excluded. In a small case series of 8 patients with metastatic uveal melanoma, anti-PD-1 therapy produced objective tumor responses (CR, n = 1; PR, n = 2; stable disease, n = 1), suggesting its potential as a therapeutic option in this patient population.[37] In an analysis of 56 patients with metastatic uveal melanoma who received anti-PD-1/PD-L1 antibodies (pembrolizumab, 38 [68%]; nivolumab, 16 [29%]; atezolizumab, 2 [4%]), durable objective responses (PRs) and stable disease ≥ 6 months were observed in 2 (3.6%) and 5 (9%) patients, respectively.[38] Median PFS (2.6 months) and OS (7.7 months) were also modestly improved. Further studies are needed to clarify the role of anti-PD-1 therapy in the treatment of metastatic uveal melanoma. Several ongoing clinical trials are investigating the use of nivolumab in combination with ipilimumab in this patient population: Phase II Multicenter, Non-Randomized, Open-Label Trial of Nivolumab in Combination with Ipilimumab in Subjects with Previously Untreated Metastatic Uveal Melanoma (NCT02626962) and Phase II Study of Nivolumab in Combination with Ipilimumab for Uveal Melanoma (NCT01585194).

**RT and immunotherapy** are both well-established treatments for malignant disease. RT is proven to provide local tumor control. The recent success with novel immunomodulatory agents has brought immunotherapy into the forefront of clinical practice for the treatment of many tumor types. RT has traditionally been thought to mediate tumor regression through direct cytotoxic effects. However, it is now known that RT also alters the local tumor microenvironment to affect both local and systemic antitumor immune responses. There is growing evidence that the rational integration of RT with the expanding armamentarium of clinically approved immunotherapeutics can yield potent antitumor responses exceeding those of either therapy alone.[39] The beneficial effects of RT in cancer patients extend beyond direct tumor cell cytotoxicity. Delivery of localized radiation to tumors often leads to systemic responses at distant sites, a phenomenon known as the abscopal effect, which has been attributed to the induction and enhancement of endogenous antitumor innate and adaptive immune responses. The mechanisms surrounding the abscopal effect are diverse and include trafficking of lymphocytes into the tumor microenvironment, enhanced tumor recognition and killing via upregulation of tumor antigens and antigen presenting machinery, and induction of positive immunomodulatory pathways.[40] Cytokines play an important role

in the abscopal effect. In one case, a Japanese patient receiving RT for thoracic vertebral bone metastasis experienced spontaneous regression of an unrelated hepatocellular carcinoma. Pre- and post-analyses of serum cytokine levels revealed a marked elevation in tumor necrosis factor-α (TNF-α) following RT, suggesting that abscopal-mediated regression may involve such cytokines as part of the host immune response.[41] RT-induced interferon (IFN)-β has been shown to enhance T cell-dependent tumor regression by increasing the cross-priming capacity of tumor-infiltrating dendritic cells (DCs) *in vivo*. This effect can be mimicked by exogenous IFN-β delivery to tumor tissues.[42] Immune cell mediation of the abscopal effect is supported by the observation that exogenous administration of chemokines following local RT enhances tumor cell killing at distal sites. This abscopal effect was tumor-type independent and involved the infiltration of CD8+ and CD4+ lymphocytes and NK1.1+ natural killer (NK) cells into the tumor sites of mice.[43] The abscopal effect remains an active area of investigation in the immunotherapy field. CTLA-4, a negative regulator of cytotoxic CD8+ T cells, has been targeted as a means to activate antitumor immune CTLs. CTLA-4 blockade has been shown to decrease the threshold of activation of endogenous tumor-reactive T cells in mouse xenografts.[44] Local RT and CTLA-4 blockade have recently been shown to significantly reduce the motility of tumor infiltrating lymphocytes (TILs) at tumor sites, thereby allowing TILs to engage in stable interactions with tumor targets.[45] The NK group 2, member D (NKG2D) ligand retinoic acid early inducible-1 is upregulated in irradiated neoplastic cells; interaction with its receptor, NKG2D, on CTLs costimulates and enhances tumor cell killing. T cell receptor, NKG2D, and CTLA-4-transduced signals contribute to the stability of the immunological synapse. Their association appears to be mediated in part by increased antibody responses to the multiple tumor antigens released after RT.[46]

Multiple factors contribute to the development of an abscopal effect. The abscopal effect involves the interplay of irradiation and induction of adaptive immune responses leading to tumor cell elimination at distant sites. Antitumor CTL responses, which represent the outcome of an abscopal effect primed by irradiated tumor cells, seem to play a significant role in RT-induced antigen-specific immunity.[47] Further evidence that antigen-specific T cells are elicited after RT is borne out by studies demonstrating a significant CD8+ T cell-mediated reduction in systemic tumor burden after local ablative RT.[48] T cell priming following RT-mediated tumor cell death has been postulated to occur through DC cross presentation of released tumor antigens in draining lymph nodes, leading to primary or metastatic tumor rejection.

Antigen presentation by DCs seems to be crucial to RT-induced CD8+ T cell-dependent antitumor immunity. Antigens can be endogenously or exogenously loaded onto major histocompatibility complex (MHC) class I molecules. RT seems to differentially affect these two antigen presentation pathways. Presentation of endogenous antigens is blocked in irradiated DCs, whereas presentation of exogenously pulsed peptide antigens is enhanced in irradiated DCs, leading to favorable antitumor T cell responses.[49] RT dose dependently facilitates cell surface expression of MHC class I molecules by three different mechanisms: (1) induction of protein unfolding and degradation to increase the intracellular peptide pool; (2) enhancement of protein synthesis to increase the intracellular peptide pool; and (3) generation of radiation-specific peptide antigens to increase the diversity of the intracellular peptide pool. By increasing the quantity and/or diversity of the peptide pool, RT leads to an overall increase in the number and density of surface peptide/MHC class I complexes expressed on murine DCs.[50] In some cases, RT upregulates cancer-testis antigens, a class of potentially immunogenic tumor rejection antigens, which can be targeted with adoptive T cell therapy and other antigen-specific immune-based approaches.[51] One significant hurdle faced by T cell-based immunotherapies is downregulation of MHC genes, which may represent an important mechanism by which tumor cells, especially those breaching the interface between normal and malignant tissues, evade host immune surveillance.

Regulatory T cells (Tregs) are more radioresistant than conventional effector T cells[52] and may be over-represented in RT-treated patients compared with RT-naïve patients.[53] Radiation has been shown to upregulate transforming growth factor-β and adenosine A2A in head and neck squamous cell carcinoma patients.[54] This can provide both a growth and survival advantage to Tregs[55], thereby suppressing the potential beneficial antitumor effects of RT. Strategies to eliminate or suppress the number and activity of Tregs such as adjusting RT dose and schedule would enhance RT-induced antitumor responses.

In most cases, abscopal effects were observed in patients with lymphoid malignancies wherein, radiation or treatment of local disease led to regression in distant unirradiated sites.[56-58] However, notable cases have been observed in Merkel cell carcinoma,[59] advanced uterine cervical carcinoma,[60] and hepatocellular carcinoma. Irradiation of affected lymphoid sites may be more likely to incite systemic immunity because of the higher likelihood of immune effectors trafficking through these regions and encountering released antigen. However, irradiation of affected visceral sites including bone, skin, and parenchyma has also been shown to induce abscopal effects.[41, 60, 61] In cancer therapies, some notions of metastasis and recurrence may be explained using oligometastases and oligo-recurrence. Oligometastases is the state capable of achieving long-term survival or cure with local therapy despite active primary lesions. On the other hand, oligo-recurrence is the notion that metastatic and recurrent lesions could be treated with local therapy since the primary lesions have been controlled.[62-64]. SBRT provides a treatment option for oligometastases by enabling the delivery of high-dose (HD) oligo-fractionated radiation to deep-seated tumors while minimizing damage to normal tissues.[65] This HD ablative RT can also be employed in combination strategies such as adoptive cell and anti-CTLA-4 therapies. Administration of autologous DCs, produced *ex vivo* through autologous leukapheresis-derived monocytes, can also boost immune responses presumably by facilitating the presentation of tumor antigens released during RT.[66] In clinical trials, the addition of SBRT to HD IL-2 has been shown to be highly effective in patients with metastatic melanoma and renal cell cancer and represents a clinically tenable strategy given that HD IL-2 is approved for use in these malignancies. The presence of an elevated effector memory CD4+ T cell population in the peripheral blood was associated with a clinical response in these patients.[67]

**Viral vectors** have also been shown to induce antitumor immune responses. Completed and ongoing clinical trials have shown that adenovirus-mediated (ADV) expression of herpes simplex virus (HSV) thymidine kinase (tk) followed by ganciclovir (GCV) therapy (ADV/HSV-tk + GCV) has a favorable toxicity profile and antitumor activity in prostate cancer. Furthermore, this system has been shown to direct systemic antitumor activity in several experimental cancer models, including prostate cancer and thus, may serve as the basis for in situ immunomodulatory gene therapy. In a mouse model of prostate cancer, NK cells were shown to mediate the antimetastatic activity of ADV/HSV-tk + GCV. To enhance its antitumor activity, ADV/HSV-tk + GCV has been combined with ADV/interleukin (IL)-12. IL-12 increases NK cell proliferation and cytotoxicity. ADV/HSV-tk + GCV + ADV/IL-12 combination therapy demonstrated superior local and systemic growth suppression compared with either therapy alone. Importantly, when the metastatic tumor burden was increased to an extent that negated the growth-suppressive activity directed by ADV/HSV-tk + GCV or ADV/IL-12 alone, the combination therapy continued to demonstrate significant growth suppression. Examination of TILs showed enhanced NK lytic activity with the combination therapy.[68] Suicide gene therapy using HSV-tk + GCV therapy is being explored for the treatment of a wide variety of cancers. HSV-tk phosphorylates GCV, converting it to a non-diffusible nucleoside analog that terminates DNA synthesis, causing cell death.[69] However, gene therapy approaches for metastatic cancer are divided between those that deliver genes hematogenously to disseminated lesions, requiring methods of tissue-restricted gene expression or specific tissue targeting by the delivery vector, and those that manifest systemic antitumor capabilities following local gene expression, such as gene-modified immunotherapy.[70] With regard to the HSV-tk system, the generation of immunologic activity has been suggested by numerous investigators to be an important aspect of therapy that may be exploited in the treatment of disseminated tumor lesions.

Several avenues of study have laid the groundwork for invoking the importance of an immune response with this therapy. HSV-tk + GCV-treated tumors contain areas of necrosis highlighted by infiltration of macrophages and CD4+ and CD8+ T-cells.[71-74] Furthermore, within treated tumors are detectable levels of the cytokines IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and granulocyte macrophage colony-stimulating factor (GM-CSF) in a fashion consistent with a T-helper type-1 (Th1; cellular based) response profile.[74, 75] Functional antitumor roles for these immune effectors have been suggested to impact within a treated tumor in the form of a local bystander effect and on synchronously growing non-transduced tumors or challenge tumor-cell injections in the form of a distant bystander effect.[71, 76-79] Further supportive evidence has noted a significant loss of growth suppression in

immunocompromised hosts.[72, 75, 76] HSV-tk + GCV may induce immune-related activities through necrosis-mediated exposure of putative tumor antigens to the cytokine-stimulated lymphocytic infiltrate. However, for the most part, direct demonstration of effector cell induction and its relevance to *in vivo* responses is lacking. In an orthotopic model of mouse prostate cancer, ADV/HSV-tk + GCV therapy was found not only to inhibit local tumor growth but also to suppress spontaneous metastatic activity.[77] Furthermore, following surgical removal of a treated subcutaneous tumor, systemic activity against a tumor-cell challenge injection of parental cells via the tail vein was induced by ADV/HSV-tk + GCV treatment of the primary tumor.[77] Direct evidence of HSV-tk + GCV-mediated induction of antitumor lymphocytes was provided by a serial assay demonstrating the lytic activity of TILs isolated from HSVtk + GCV-treated orthotopic tumors against parental tumor cells *in vitro*.[78] These studies demonstrated the induction of NK cells by ADV/HSV-tk + GCV treatment. Gene therapy performed in the absence of NK cells, but not T cells, resulted in a modest but significant loss of HSVtk + GCV-directed growth suppression within the primary tumor and complete abrogation of systemic activities.[78] Therefore, HSV-tk + GCV therapy appears to induce NK cells to impact local and remote tumor growth. Enhancement of NK cell activity may improve therapeutic response to ADV/HSV-tk + GCV.

Viral vectors have been increasingly studied as potential antitumor agents. With their ability to invade and replicate within target cells, viruses have been utilized as oncolytic agents to directly lyse tumor cells. Viruses can also deliver their genetic payload into infected cells, allowing for the repair of defective tumor suppressor genes, disruption of oncogenic pathways, and production of cytokines that activate the immune system. Furthermore, viruses encoding tumor-associated antigens can infect DCs to trigger tumor-specific immune responses. The ability to engineer viruses with high levels of tumor specificity and efficient rates of infection has enhanced the safety profile of these agents, making viral vector-mediated gene therapy, either alone or in conjunction with more conventional therapies, a viable option for cancer therapy.[79]

## 2. Significance and rationale and study purpose

The significance of this proposal is to establish the superior efficacy of RT in combination with other immunomodulators for mounting an effective antitumor immune response. RT in combination with immunomodulators can produce significant local control and induce antitumor responses at distant sites by triggering and enhancing endogenous cellular immune responses. Here, we propose to manipulate the antigen-specific immune response by vaccination in combination with RT followed by immunomodulatory therapy with the anti-PD-1 compound nivolumab.

**Current data strongly indicate the potential of ADV/HSV-tk in situ gene therapy plus SBRT to enhance the response to immunomodulatory therapy with nivolumab. This treatment regimen may have applicability against a wide range of solid tumors.**

Data originating from these studies will identify novel mechanisms involved in NSCLC and uveal melanoma, the antitumor effects of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT-containing regimens, and alternative pathways and/or strategies to induce the abscopal effect and avoid or overcome drug resistance and undesired toxicity effects. It is expected that the results from this study will lead to better and alternative treatment options not only for patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma but also those with other solid malignancies.

### 2.1 Innovation

Nivolumab, an anti-PD-1 monoclonal antibody, has been established as a successful treatment paradigm in metastatic squamous and non-squamous cell NSCLC. Some small studies have suggested the potential therapeutic efficacy of PD-1 blockade in patients with metastatic uveal melanoma.[37,38] Viral vector-based gene therapy such as ADV/HSV-tk + GCV has also been shown to induce antitumor immune activity by several mechanisms including enhancing NK proliferation and cytotoxicity, boosting cytokine stimulatory activity, and increasing lymphocytic infiltrate. In addition, RT has been shown to augment endogenous antitumor innate and adaptive immune responses. Thus, RT in combination with immunomodulators can produce significant local control and induce antitumor responses at distant sites by triggering and amplifying endogenous cellular immune

responses. Here, we propose a rational combination of ADV/HSV-tk in situ gene therapy plus SBRT followed by immunomodulatory therapy with nivolumab for the treatment of metastatic and non-squamous squamous NSCLC and metastatic uveal melanoma. If proven to be efficacious and safe, this novel therapeutic approach will provide an improved treatment option for patients with metastatic squamous or non-squamous NSCLC whose disease has progressed on or after platinum-based chemotherapy or on or after immune checkpoint therapy and for patients with metastatic uveal melanoma.

## 3. Hypothesis:

***We hypothesize that in situ administration of ADV/HSV-tk + Valacyclovir followed by SBRT to a dominant lung tumor will upregulate immunogenic neoantigens, thereby improving the systemic response to subsequent treatment with an immune checkpoint PD-1 inhibitor (nivolumab).***

We aim to demonstrate the efficacy and safety of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma.

**Research strategy general considerations:** Administration of ADV/HSV-tk + Valacyclovir in combination with SBRT before nivolumab represents a novel window of opportunity to enhance nivolumab efficacy by boosting endogenous immune-mediated antitumor activity and neoantigen expression. The aims of this study are to assess the efficacy, safety, and toxicity of this new therapeutic approach. This study will also provide a deeper understanding of the immune response and abscopal effect elicited by this therapeutic approach.

## 4. Objectives and endpoints

Primary Objective:

- To determine the ORR of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma. Both RECIST 1.1 and modified immune-related response criteria (irRC; derived from RECIST 1.1) will be used to assess treatment response (See Appendix D).

Secondary Objectives:

- To determine the DoR of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma.
- To determine the OS rate of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma.
- To determine the PFS rate of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma.
- To document the toxicities associated with ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma, as assessed by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03.
- To document the antitumor activity of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma, as assessed by both RECIST 1.1 and modified irRC (See Appendix D).

- To estimate the clinical benefit rate (CBR) of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma.

Exploratory Objectives:

- To determine the abscopal effect of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT. The primary criterion for abscopal effect evaluation will be computed tomography (CT)-based response assessment of a non-targeted lesion. The secondary criterion will be immune parameters including PD-1 and PD-L1 expression, immune infiltrates, and cytokine expression (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF).
- To measure the immune response to nivolumab in patients with squamous or non-squamous NSCLC and metastatic uveal melanoma.
- To assess the Th1 (cellular-based) response by measuring the following cytokines: IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF.
- To measure TILs before and after ADV/HSV-tk + Valacyclovir therapy.
- To explore correlative tissue and blood-based biomarkers of treatment response including but not limited to T-cell cytokine profiles (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF), TILs, PD-1 and PD-L1 expression, effector and suppressor immunocyte populations.

**Endpoints**

**Primary endpoint:** The primary endpoint will be the ORR of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma. Both RECIST 1.1 and modified irRC will be used to assess treatment response.

**Secondary endpoints:** Secondary endpoints will include a) DoR; b) OS and PFS rates; c) CBR; d) safety and toxicity (toxicity will be defined as any treatment-related death or any **≥ Grade 3 toxicity** excluding alopecia and constitutional symptoms as assessed by NCI CTCAE v4.03); e) immune-mediated antitumor activity (assessed by RECIST 1.1 and modified irRC); and f) correlative tissue and blood-based biomarkers of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab.

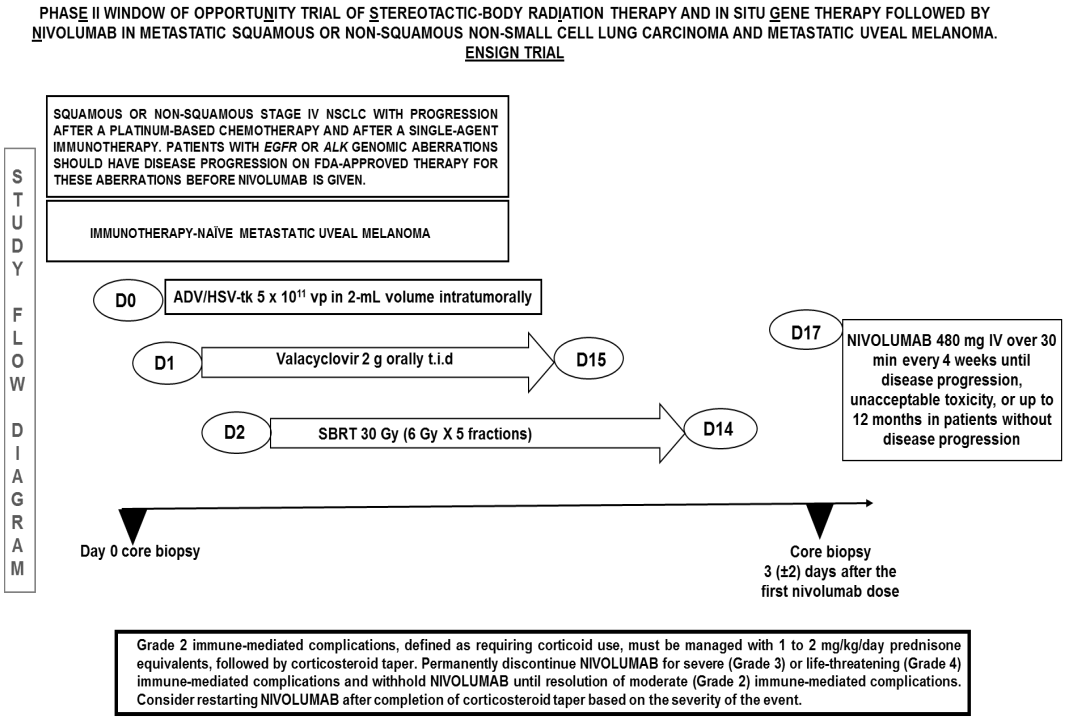
**Tumor assessments:** Tumor assessments will be performed at baseline and every eight (8) weeks thereafter until completion of the protocol-specified study treatment, and 30 days after the last dose of nivolumab. Disease status will be assessed using both RECIST 1.1 and modified irRC (assessments will be performed at specified time points). Serum alanine aminotransferase (ALT) levels will be assessed on day 1 of each cycle. The ALT level must be within the acceptable range before administering the study drug combination, and dosing will be discontinued either temporarily or permanently for any patient who has a severe cytokine reaction, elevated ALT level (> 5 × upper limit of normal [ULN]), or any other Grade 4 or greater adverse effect.

## 5. Study design

### 5.1 Description of study design

This is a phase II window of opportunity study evaluating the efficacy and toxicity of SBRT and in situ gene therapy followed by nivolumab in metastatic squamous and non-squamous NSCLC and metastatic uveal melanoma.

Figure 1: Study design




## 6. Treatment

**ADV/HSV-tk** ($5 \times 10^{11}$ viral particles) in a 2-mL total volume will be injected intratumorally on day 0 of the study.
**Valacyclovir** will be orally administered at a dose of 2 g three times daily (t.i.d.) for 14 days. For patients with serum creatinine level between 1.6−2.0 × ULN, valacyclovir dose will be reduced 50% (i.e., 1 g t.i.d.). Valacyclovir treatment will be administered 24 hours after the gene vector injection from day 1 to day 15 of the study.
**SBRT** of 30 gray (Gy; 6 Gy X 5 fractions) will be administered over 2 weeks from day 2 to day 14 of the study.
**Nivolumab** (480 mg) will be administered intravenously over 30 minutes every 4 weeks (± 1 day) starting on day 17 of the study and continuing until disease progression, unacceptable toxicity, or up to 12 months in patients without disease progression.
**Immune-mediated complications of nivolumab** will be defined as immunological reactions requiring the use of corticosteroids. Grade 2 or greater immune-mediated complications must be managed with 1 to 2 mg/kg/day prednisone equivalents, followed by corticosteroid taper. Permanently discontinue nivolumab for severe (Grade 3) or life-threatening (Grade 4) immune-mediated complications and withhold nivolumab until resolution of moderate (Grade 2) immune-mediated complications. Based on the severity of the event, consider restarting nivolumab after completion of corticosteroid taper.

Patients whose treatment is interrupted or permanently discontinued due to an AE including abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4-week intervals until resolution or stabilization of the event, whichever comes first. Dose interruptions should be reported on the appropriate Dosage Administration case report form (CRF). The maximum time allowed for toxicity-related treatment interruption is 21 days (3 weeks) from the intended dosing day. If interruption is > 3 weeks, the patient must be discontinued from the study treatment. However, the patient will continue to be followed for toxicity.

**7. Population**

Metastatic NSCLC: Male or female patients aged ≥18 years with histologically or cytologically confirmed metastatic squamous or non-squamous NSCLC whose disease has progressed after a platinum-based chemotherapy and after a single-agent immunotherapy. Patients with *EGFR* or *ALK* genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving nivolumab. The investigator or designee must ensure that the patient meets all the inclusion and none of the exclusion criteria before being offered enrollment in the study.

Metastatic Uveal Melanoma: Male or female patients aged ≥18 years with histologically or cytologically confirmed metastatic uveal melanoma that is immunotherapy naïve.

**Study Duration:**

After administration of ADV/HSV-tk + Valacyclovir followed by SBRT, nivolumab will be given every 4 weeks from day 17 until disease progression, unacceptable toxicity, or up to 12 months in patients without disease progression. Patients will be followed up for 6 months or until disease progression, whichever occurs first. Patients will be assessed for AEs up to and including 30 days after the last treatment dose.

**Safety Criteria:**

A screening medical history and physical exam will be performed (baseline symptom-medical history and physical examination are not required if the screening was conducted within 7 to 28 days prior to day 0 of ADV/HSV-tk). Patients presenting with any medical history, physical exam, or laboratory abnormality that, in the in the opinion of the treating physician, would put their safety at risk will be excluded. A window of ± 5 days is allowed for study visits and assessments (except as otherwise specified). A physical exam will be performed on days 0 and 17; every 4 weeks thereafter until completion of the protocol-specified treatment; and at end of treatment (EOT). Vital sign (blood pressure, heart rate, and oral temperature) measurements will be performed at each physical exam. A 12-lead electrocardiogram (ECG) will be performed at screening, at EOT, and when clinically indicated. Multigated acquisition (MUGA) scan or echocardiogram (ECHO) will be performed when clinically indicated. The same method (ECHO or MUGA scan) must be used throughout the duration of the study. A blood sample for complete blood count (CBC) with platelet count and differential white blood cell (WBC) count will be obtained at screening, on days 0 and 17, every 4 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. If a patient is found to have an absolute neutrophil count (ANC) <1.5 x $10^{\wedge 9}$/L, platelet count < 100 x $10^{\wedge 9}$/L, or both, the CBC with differential should be repeated at least every other day until the ANC and platelet count have exceeded these values. A blood sample for clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, chloride, blood urea nitrogen [BUN], creatinine, total bilirubin, alkaline phosphatase, aspartate transaminase [AST], and ALT) and evaluation of magnesium and lactate dehydrogenase will be obtained at screening, on days 0 and 17, every 4 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. Prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT) testing will be performed at screening, on day 17, every 4 weeks thereafter until completion of the protocol-specified treatment, and at EOT. For women of childbearing potential (WOCBP), the results of a serum β-human chorionic gonadotropin (β-hCG) pregnancy test must be negative within 7 days of the administration of the first treatment dose. If the screening serum β-hCG pregnancy test is performed more than 7 days before ADV/HSV-tk dosing, it must be repeated at baseline, with results known to be negative prior to the administration of the first dose of the study treatment. Serum β-hCG pregnancy testing is to be repeated as clinically indicated. Laboratory tests may be done more frequently if medically indicated. Patients will be assessed for AEs and SAEs from informed consent signing up to and including 30 days after the last treatment dose. Study treatment-related AEs occurring beyond 30 days after the last dose of nivolumab and any study patient death should also be reported. Toxicity will be defined as any treatment-related death or any ≥ Grade 3 toxicity excluding alopecia and constitutional symptoms as assessed by NCI CTCAE v4.03. Patients whose treatment is interrupted or permanently discontinued due to an AE including

abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4-week intervals until resolution or stabilization of the event, whichever comes first. The maximum time allowed for toxicity-related treatment interruption is 21 days from the intended dosing day. If the interruption is > 3 weeks, the patient must be discontinued from the study treatment. However, the patient will continue to be followed for toxicity. Brain magnetic resonance imaging (MRI) will be performed at screening. Relapsed/refractory and/or metastatic patients will be evaluated with CT scan of the thorax, abdomen, and pelvis at screening, every eight (8) weeks thereafter until completion of the protocol-specified treatment and/or at the discretion of the treating physician, at EOT, and as clinically indicated. For patients with equivocal CT scan results, positron emission tomography (PET) scan will be performed. In patients with accessible tumor, biopsies will be conducted at day 0, after the first nivolumab dose (day 17 ± 2 days), and if the patient progresses while on treatment, before the patient starts the new treatment. Blood samples for correlative studies will be collected at screening, after the first dose (± 2 days) of nivolumab, and at the end of Cycle 2 of nivolumab. An additional sample will be collected from subjects whose disease progresses while on treatment. Blood samples will be collected into standard vacutainer tubes (3 green top tubes). Samples will be evaluated for profile of circulating suppressor and effector immunocytes and cytokines.

## 8. Inclusion and exclusion criteria

Patients must have baseline evaluations performed prior to treatment start and must meet all inclusion criteria and none of the exclusion criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule, required evaluations, and all regulatory requirements for informed consent. Written informed consent must be obtained from the patient prior to enrollment. The criteria below apply to all patients enrolled onto the study unless otherwise specified.

### 8.1 Inclusion criteria:

Patient must meet all of the following criteria:

- Male or female ≥18 years of age.
- Histologically or cytologically confirmed stage IV metastatic squamous or non-squamous NSCLC that has progressed after a platinum-based chemotherapy and after a single-agent immunotherapy **OR** histologically or cytologically confirmed metastatic uveal melanoma that is immunotherapy naïve.
- Evaluable or measurable disease as per RECIST 1:1, a target lesion of suitable diameter (at least 5 mm) for SBRT, and a non-target lesion of at least 1 cm in diameter for abscopal effect evaluation.
- ≥ 4 weeks since any major surgery.
- A 2-week washout period post any prior systemic anticancer therapy, RT, and/or investigational therapy is required prior to trial entry. Subject should be adequately recovered from the acute toxicities of any prior therapy.
- Life expectancy ≥ 6 months.
- ECOG performance status of 0–1.
- Adequate bone marrow function:
  - ANC ≥ 1.5 x 10^9/L (without granulocyte colony stimulating factor support within 2 weeks of laboratory test used to determine eligibility)
  - Platelets ≥ 100 x 10^9/L (without transfusion within 2 weeks of laboratory test used to determine eligibility)
  - Hemoglobin ≥ 8 g/dL (without blood transfusion)
  - WBC count > 2,500/μL and < 15,000/μL
  - Lymphocyte count ≥ 500/μL
- Adequate liver function:
  - Serum bilirubin ≤ 1.0 × ULN (patients with known Gilbert's disease who have serum bilirubin level ≤ 3 × ULN may be enrolled)

- Serum transaminases (AST or ALT) activity ≤ 2.5 × ULN with normal alkaline phosphatase (patients with liver metastases ≤ 5 x ULN) OR AST and ALT ≤ 1.5 × ULN with an alkaline phosphatase > 2.5 × ULN

- INR or PT and aPTT ≤ 1.5 × ULN (unless patient is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants);
- Adequate renal function: serum creatinine <2 x ULN.
- WOCBP must have a negative serum pregnancy test within 7 days prior to the administration of the first study treatment. Women must not be lactating.
- WOCBP and men must practice an effective method of birth control (See Section 12.5).
- Signed informed consent to participate in the study must be obtained from patients after they have been fully informed of the nature and potential risks of the study by the investigator (or his/her designee) with the aid of written information.
- Willing to provide biopsies as required by the study.

**8.2 Exclusion criteria:**

- Prior treatment with gene therapy.
- Any immunotherapy within 2 weeks of study treatment start (NSCLC cohort only).
- Prior treatment with immunotherapy (uveal melanoma cohort only)
- Patients with *EGFR* or *ALK* genomic tumor aberrations that have not received any FDA-approved therapy for these aberrations (NSCLC cohort only).
- Oxygen-dependent chronic obstructive pulmonary disease (NSCLC cohort only).
- Patients requiring oral prednisone for emphysema management (NSCLC cohort only).
- History of liver disease, such as cirrhosis or active/chronic hepatitis B or C.
- History of or current alcohol misuse/abuse within the past 12 months.
- Known gallbladder or bile duct disease (i.e., infection or cholecystitis) or acute or chronic pancreatitis.
- Major surgery within 4 weeks prior to study enrollment.
- Uncontrolled brain or leptomeningeal metastases or brain or leptomeningeal metastases requiring continued glucocorticoid treatment. Patients who have been treated at least 4 weeks prior to enrollment and have a CT or MRI scan of the brain showing no evidence of disease progression within 4 weeks of enrollment are eligible.
- Congestive heart failure: New York Heart Association class III or IV heart failure or unstable angina.
- History of syncope or family history of idiopathic sudden death.
- Sustained or clinically significant cardiac arrhythmias including sustained ventricular tachycardia, ventricular fibrillation, clinically significant bradycardia, advanced heart block (Mobitz II or higher atrioventricular nodal block), prolonged heart rate-corrected QT interval (longer than 470 milliseconds), or history of acute myocardial infarction. (The QT interval is the time between the start of the Q wave and the end of the T wave in the cardiac electrical cycle)
- Concomitant disease(s) that could prolong QT such as autonomic neuropathy (caused by diabetes or Parkinson's disease), human immunodeficiency virus (HIV), cirrhosis, uncontrolled hypothyroidism, or cardiac failure.
- Presence of active or suspected acute or chronic uncontrolled infection or history of immunocompromise, including a positive HIV test result.
- Any severe and/or uncontrolled medical conditions or other conditions that could affect patient participation in the study such as severely impaired lung function, any active (acute or chronic) or uncontrolled infection/disorders, and nonmalignant medical illnesses that are uncontrolled or whose control may be jeopardized by the study therapy.

- Known or suspected allergy or hypersensitivity to any component of nivolumab or its analogues or any component of the proposed regimen (gene vector/Valacyclovir).
- Inability to swallow food or any condition of the upper gastrointestinal tract that precludes administration of oral medications (Valacyclovir). Any active malignancy except for non-melanoma skin cancer or in situ cervical cancer or treated cancer from which the patient has been continuously disease free for more than 5 years.
- Pregnant or breastfeeding women or women/men able to conceive and unwilling to practice an effective method of birth control. WOCBP must have a negative serum pregnancy test within 7 days prior to the administration of the first study treatment.
- Unwilling or unable to comply with the study protocol.

## 9. Treatment materials

### 9.1 ADV/HSV-tk

**Replication-defective recombinant adenovirus vector.**

**The viral vector proposed here has been used for multiple ongoing Investigational New Drugs (INDs), including BB-IND 6371, 6636, 6599, and 7311. The vector is a first-generation adenoviral construct based on an adenovirus serotype-5 backbone.**

The therapeutic potential of HSV-tk gene therapy is greatly enhanced by a "bystander effect" in which cytotoxicity is conferred to non-transduced neighboring cells. *In vivo* bystander effects are likely due to a combination of host immunological responses and to gap junction-mediated transport of phosphorylated prodrug metabolites to surrounding cells.

Many laboratories have shown the *in vitro* susceptibility of mouse and human prostate cancer cells to HSV-tk/prodrug treatment. Our group has evaluated the effects of ADV-tk + GCV on *in vivo* tumor growth and survival in mouse prostate cancer models.[80] An adenoviral vector carrying the HSV-tk gene under the control of the Rous sarcoma virus promoter was administered by direct intratumoral injection.[80-82] Tumor growth was significantly delayed in the ADV-tk + GCV group compared with the control groups (ADV-tk + saline and ADV-β-galactosidase [control vector] + saline or GCV).[80] Tumor volume in the ADV-tk + GCV-treated group was 16% of that in the combined control groups. However, with longer follow-up, ADV-tk + GCV-treated tumors began to grow at a rate similar to control tumors. Mean survival was 14.2 ± 0.6 days for the combined control groups and 21.2 ± 1.3 days for the treatment group ($P < 0.001$ by Mantel-Cox log-rank analysis). Animal studies have also shown that ADV-tk treatment can protect against metastases. In an orthotopic model, the spontaneous metastasis rate was significantly lower for the ADV-tk + GCV treatment group compared with the pooled control groups (12.5% [2/16] vs. 71.4% [10/14]; $P = 0.0032$ by Fisher's Exact test).[83] Similar results were obtained in experimental metastasis models (i.e., tail vein injection of tumor cells). HSV-tk/GCV gene therapy in combination with radiation significantly prolonged survival compared with either therapy alone and sham treatment (22.9 ± 1.34 days vs. 16.9 ± 0.84 days and 11.3 ± 0.77 days, respectively; $P < 0.001$ by log-rank and Wilcoxon tests).[83]

Based on extensive efficacy and toxicity studies in mice, cotton rats and non-human primates[81, 84] our group designed a dose escalation phase I clinical study to evaluate the potential toxicity of ADV/HSV-tk in humans (Regulatory Affairs Certification [RAC] protocol # 9601-144). The study was evaluated and approved by the local (institutional review board [IRB] and institutional biosafety committee) and national (RAC and FDA) regulatory bodies. Subjects included men with locally recurrent prostate cancer after RT. Vector particles ($2 \times 10^9$ to $2 \times 10^{12}$) were administered in a single intratumoral injection guided by ultrasound imaging. Three patients achieved partial responses, and only occasional transient toxicities were observed (Phase I Study of Adenoviral Vector Delivery of the HSV-tk Gene and the Intravenous Administration of Ganciclovir in Men with Local Recurrence of Prostate Cancer after Radiation Therapy, National Institutes of Health-Office of Recombinant DNA Activities # 9601-144).[85]

Based on our favorable preclinical and clinical trial data, a phase I-II study was designed to analyze the efficacy and toxicity of HSV-tk gene therapy in combination with RT in prostate cancer patients.[86] In this study, intravenous GCV was replaced with valacyclovir (Valtrex, Glaxo Wellcome), an orally administered medication that is rapidly and almost completely metabolized in the liver to acyclovir. The trial consisted of 3 treatment arms: Arm A, low-risk patients treated with HSV-tk/valacyclovir + RT; Arm B, high-risk patients treated with the same regimen as Arm A with the addition of hormonal therapy; and Arm C, patients with stage D1 (positive pelvic lymph nodes) who received the same regimen as Arm B with the addition of 45 Gy to the pelvic lymphatics. Fifty-nine patients (29 in Arm A, 26 in Arm B, and 4 in Arm C) completed the trial. The median age was 68 years (range: 39–85 years). The median follow-up for the entire group was 13.5 months (range: 1.4–27.8 months). Only Arm A patients were observed to have an increase in prostate specific antigen (PSA) on day 14, which then declined appropriately. All patients in Arm A (median follow-up: 13.4 months) and Arm B (median follow-up: 13.9 months) had biochemical control at last follow-up. Three patients in Arm C (with pretreatment PSA of 335, 19.6, and 2.5 ng/mL and a combined Gleason score of 8, 9, and 9 involving all biopsy cores) had biochemical failure at 3, 3, and 7.7 months. Two patients had distant failure in bone and 1 patient in the para-aortic lymph nodes outside the radiation portal. Six to twelve prostate biopsies performed in these 3 patients revealed no evidence of residual carcinoma. In Arm A, biopsy showed no evidence of carcinoma in 66.7% (18/27), 92.3% (24/26), 91.7% (11/12), 100% (8/8), and 100% (6/6) of patients at 6 weeks, 4 months, 12 months, 18 months, and 24 months after treatment, respectively. In Arm B, biopsy showed no evidence of carcinoma in 96% (24/25), 90.5% (19/21), 100% (14/14), 100% (7/7), and 100% (2/2) of patients at 6 weeks, 4 months, 12 months, 18 months, and 24 months after treatment, respectively. This was the first reported trial of its kind in the field of prostate cancer that aimed to expand the therapeutic index of RT by combining it with in situ gene therapy.[87]

**Dosage:**

ADV/HSV-tk will be administered intratumorally at 5 x $10^{11}$ virus particles on day 0. This dose is based on the toxicity results from the phase I/II study of ADV-tk gene therapy in combination with chemoradiation in patients with pancreatic adenocarcinoma.[77] If toxicity occurs, ADV/HSV-tk dose will be reduced to 5 x $10^{9}$ vector particles per injection. If toxicity occurs with this dose modification, the dose will be further reduced to 5 x $10^{6}$ virus particles per injection.

**Delivery:**

For the NSCLC cohort, the tumor will be identified by PET/CT by the radiation oncologist. The procedure will be managed under endoscopic ultrasound (EUS) guidance. In rare cases where the tumor cannot be accessed by EUS, a CT-guided injection will be performed. For the uveal melanoma cohort, the procedure will be managed under ultrasound or CT guidance. EUS will be performed with the patient under sedation. Patients will be monitored until the sedation has worn off. For CT-guided injections, local anesthesia will be used and patients will be monitored for several hours after the procedure.

**Warnings and precautions:**

Significant toxicity has not been observed with the administration of ADV/HSV-tk gene therapy alone or in combination with RT or chemotherapy in previous clinical trials.[85, 88-90] Fever may occur due to the local inflammatory and immune response to ADV/HSV-tk, which will be treated with acetaminophen as necessary. If local inflammation is extensive or infection develops, Valacyclovir will be stopped and antibiotics and/or anti-inflammatory therapy will be given as appropriate. Other potential side effects of ADV/HSV-tk gene therapy include increased creatinine, increased liver enzymes (ALT, AST, alkaline phosphatase, and gamma-glutamyl transferase), increased bilirubin, cellulitis, and thrombocytopenia.

### 9.2 Valacyclovir[91]

**Proper name:** Valacyclovir (as valacyclovir hydrochloride)
**Chemical name:** L-valine, 2-[(2-amino-1, 6-dihydro-6-oxo-9H-purin-9-yl)
**Molecular formula:** C13H20N6O4•HCl

**Molecular mass:** 360.80

**Structural formula:**

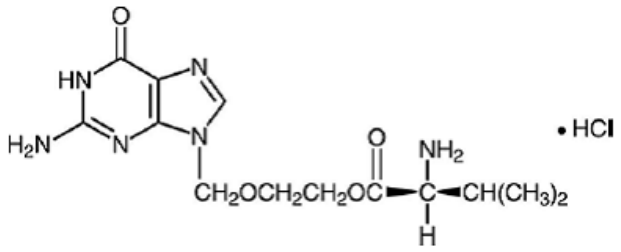


Figure 2

**Physicochemical properties:**
Valacyclovir hydrochloride is a white to off-white powder with a maximum solubility in water of 174 mg/mL at 25°C. Valacyclovir hydrochloride has no distinct melting point. It undergoes rapid decomposition above 200°C. A saturated solution ($4.82 \times 10^{-1}$) of valacyclovir HCl in distilled water has a pH of 3.5 at 25°C. The pKa values of valacyclovir are pKa1 = 1.90, pKa2 = 7.47, and pKa3 = 9.43.

**Mechanism of action:**
Valacyclovir is the L-valyl ester and a prodrug of the antiviral drug acyclovir. Valacyclovir hydrochloride is rapidly converted to acyclovir, which has *in vitro* and *in vivo* inhibitory activity against human herpes viruses including HSV-1, HSV-2, and varicella-zoster virus (VZV).
The inhibitory activity of acyclovir is highly selective due to its unique affinity for HSV and VZV-encoded tks. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. Once incorporated, acyclovir irreversibly binds to viral DNA polymerase, effectively inactivating the enzyme. Acyclovir triphosphate is a potent inhibitor of all the human herpes virus DNA polymerases studied. Acyclovir is virtually inactive in uninfected cells, because it is preferentially taken up and selectively converted to the active triphosphate form by herpes virus-infected cells. Additionally, the tk of uninfected cells does not effectively use acyclovir as a substrate, and cellular α-DNA polymerase is less sensitive to the effects of acyclovir than viral DNA polymerase.
A combination of the tk specificity, competitive inhibition of DNA polymerase, and incorporation and termination of the growing viral DNA chain results in the inhibition of herpes virus replication. No effect on latent non-replicating virus has been demonstrated. Inhibition of viral replication reduces the period of viral shedding, which limits the degree of spread and level of pathology and facilitates healing. The pain of shingles is related to viral damage to neurons, which takes place during viral replication.

**Pharmacokinetics:**
Absorption: After oral administration, valacyclovir hydrochloride is rapidly absorbed from the gastrointestinal tract and nearly completely converted to acyclovir and L-valine by first-pass intestinal and/or hepatic metabolism. The absolute bioavailability of acyclovir after administration of valacyclovir hydrochloride is 54.5% ± 9.1% as determined following a 1000-mg oral dose of valacyclovir hydrochloride and 350-mg IV acyclovir dose to 12 healthy volunteers. Valacyclovir pharmacokinetics are not dose-proportional. The rate and extent of absorption decreases with increasing dose, resulting in a less than proportional increase in maximumplasma concentration (Cmax) over the therapeutic dose range and a reduced bioavailability at doses above 500 mg. Acyclovir pharmacokinetics are unaltered after multiple-dose administration.
Distribution: The binding of Valacyclovir to human plasma proteins ranges from 13.5% to 17.9%.
Metabolism: Following absorption, Valacyclovir is rapidly and nearly completely hydrolyzed to acyclovir and L-valine, an essential amino acid, by first-pass metabolism. This hydrolysis is mediated primarily by the enzyme valacyclovir hydrolase and occurs predominantly in the liver.

Elimination: The pharmacokinetic disposition of acyclovir delivered by valacyclovir is consistent with previous experience from intravenous and oral acyclovir. Acyclovir is eliminated primarily by urinary excretion of unchanged drug. In all studies of valacyclovir hydrochloride, the half-life of acyclovir typically averages 2.5 to 3.3 hours in subjects with normal renal function.

**Indications and clinical uses:**

Valacyclovir in caplet form (as valacyclovir hydrochloride) is indicated:

• For the treatment of herpes zoster (shingles).

• For the treatment or suppression of genital herpes in immunocompetent individuals and suppression of recurrent genital herpes in HIV-infected individuals.

• To reduce the risk of transmission of genital herpes with the use of suppressive therapy. Safer sex practices should be used with suppressive therapy.

• For the treatment of cold sores (herpes labialis).

Geriatrics (> 65 years of age): Use in the geriatric population may be associated with differences in safety due to age-related changes in renal function (a brief discussion can be found in the appropriate sections).

**Contraindications:**

Valacyclovir is contraindicated in patients with a known hypersensitivity or intolerance to Valacyclovir, acyclovir, or any component of the formulation.

**Warnings and precautions:**

General care should be taken to ensure adequate fluid intake in patients, particularly geriatric patients, who are at risk of dehydration.

The safety and efficacy of Valacyclovir have not been established for the treatment of disseminated herpes zoster.

The safety and efficacy of Valacyclovir have not been established in immunocompromised patients other than for the suppression of anogenital herpes in HIV-infected patients. The safety and efficacy of Valacyclovir for the suppression of recurrent anogenital herpes in patients with advanced HIV disease (CD4 cell count < 100 cells/$mm^3$) have not been established.

- Central nervous system

Central nervous system adverse reactions (e.g., agitation, hallucinations, confusion, and encephalopathy) may occur in both adult and pediatric (> 12 years of age) patients (with or without reduced renal function) and in patients with underlying renal disease who receive higher than recommended doses of Valacyclovir for their level of renal function. Elderly patients are more likely to have central nervous system adverse reactions. Use with caution in elderly patients and reduce dosage in patients with renal impairment.

- Hepatic/biliary/pancreatic

Dose modification is not required in patients with mild or moderate cirrhosis (hepatic synthetic function maintained). Pharmacokinetic data in patients with advanced cirrhosis (impaired hepatic synthetic function and evidence of portal-systemic shunting) do not indicate the need for dosage adjustment. There are no data available on the use of higher doses of Valacyclovir (≥ 4 g daily [QD]) in patients with liver disease. Caution should, therefore, be exercised when administering higher doses of Valacyclovir to these patients.

Renal Renal insufficiency and acute renal failure have been observed in patients taking Valacyclovir at the recommended dosage and/or with no previous renal conditions and may be associated with renal pain. Acyclovir, the active metabolite of Valacyclovir, is eliminated by renal clearance; therefore, the dose of Valacyclovir must be reduced in patients with renal impairment (see Dosage and administration; Patients with acute or chronic renal impairment). Geriatric patients are likely to have reduced renal function and therefore, the need for dose reduction must be considered in this group of patients. Both geriatric patients and patients with a history of renal impairment are at increased risk of developing neurological side effects and should be closely monitored for evidence of these effects. In the reported cases, these reactions were generally reversible on discontinuation of treatment. Cases of acute renal failure have been reported in patients without adequate hydration. Precipitation of acyclovir in renal tubules may occur when the solubility (2.5 mg/mL) is exceeded in the intratubular fluid. Adequate hydration should be maintained for all patients.

**Adverse reactions:**
Serious Adverse Drug Reactions
• Thrombotic thrombocytopenic purpura (TPP)/hemolytic-uremic syndrome (HUS)
• Acute renal failure
• Central nervous system effects
Adverse Drug Reaction Overview
The most frequent adverse reactions associated with the use of Valacyclovir are headache and nausea. Neurological side effects have also been reported in rare instances. Geriatric patients and patients with a history of renal impairment are at increased risk of developing these effects. In the reported cases, these reactions were generally reversible on discontinuation of treatment.
**ENSIGN Clinical Trial Adverse Drug Reactions** *Because the ENSIGN clinical trial will be conducted under very specific conditions, the adverse reaction rates observed may not reflect the rates observed in practice and should not be compared to the rates in the clinical trials of another drug.*

**Herpes Zoster:** Adverse drug reactions were not significantly different between patients receiving Valacyclovir and those receiving placebo or acyclovir in two double-blind, randomized clinical trials of treatment of herpes zoster (shingles) in immunocompetent patients. The most frequent adverse drug reactions reported in recipients of Valacyclovir are listed in Table 1.

*Table 1. Incidence (%) of Drug-Related Adverse Reactions Occurring in ≥ 1% of Patients Receiving Valacyclovir in Two Clinical Trials of Herpes Zoster*

| Adverse Drug Reaction | Herpes Zoster | | | |
|---|---|---|---|---|
| | 18–50 Years | | > 50 Years | |
| | **Valacyclovir (n=202) (%)** | **Placebo (n=195) (%)** | **Valacyclovir (n=765) (%)** | **Acyclovir (n=376) (%)** |
| Nausea | 8 | 6 | 12 | 14 |
| Headache | 11 | 8 | 8 | 7 |
| Diarrhea | 4 | 4 | 4 | 4 |
| Vomiting | 2 | 2 | 4 | 3 |
| Asthenia | 1 | 3 | 3 | 2 |
| Constipation | < 1 | < 1 | 3 | 3 |
| Abdominal pain | < 1 | 1 | 2 | 1 |
| Anorexia | < 1 | 2 | 2 | 2 |
| Dizziness | 1 | 1 | 2 | 2 |
| Dry Mouth | < 1 | 0 | 2 | 1 |
| Dyspepsia | 0 | < 1 | 2 | 1 |
| Flatulence | 0 | 0 | 1 | 1 |
| Pruritus | 1 | 0 | < 1 | 0 |

**Genital Herpes:** In two double-blind, randomized trials of treatment of recurrent genital herpes in immunocompetent patients, adverse drug reactions were not significantly different between patients receiving Valacyclovir and those receiving placebo. The most frequent adverse reactions are listed in Table 2.

*Table 2. Incidence (%) of Drug-Related Adverse Reactions Occurring in ≥ 1 % of Patients Receiving Valacyclovir in Two Clinical Trials of Treatment of Recurrent Genital Herpes*

| Adverse Drug Reaction | Valacyclovir (n=1235) (%) | Placebo (n=439) (%) |
|---|---|---|
| Headache | 11 | 9 |
| Nausea | 5 | 6 |
| Diarrhea | 4 | 4 |
| Dizziness | 2 | 2 |

| | | |
|---|---|---|
| Abdominal pain | 2 | 1 |
| Asthenia | 1 | 3 |

**Cold Sores:** Adverse drug reactions reported by patients receiving 2000 mg Valacyclovir twice daily (n=609) or placebo (n=609) for one day in clinical studies of treatment of cold sores are listed below.

*Table 3. Incidence (%) of Drug-Related Adverse Reactions Occurring in ≥ 1% of Patients Receiving Valacyclovir in Two Clinical Trials of Treatment of Cold Sores*

| Adverse Drug Reaction | Valacyclovir (n=609) (%) | Placebo (n=609) (%) |
|---|---|---|
| Headache | 9 | 5 |
| Nausea | 4 | 5 |
| Diarrhea | 3 | 3 |
| Dyspepsia | 1 | 1 |

**Abnormal hematologic and clinical chemistry findings:** In herpes zoster trials, the frequency of WBC abnormality (< 0.75 times the lower limit of normal) was 1.3% for patients receiving Valacyclovir compared with 0.6% for patients receiving placebo. This difference was not clinically or statistically significant.

In clinical studies of treatment of cold sores, the frequencies of abnormal ALT values (> 2 × ULN) were 1.8% for patients receiving Valacyclovir at the recommended clinical dose and 0.8% for patients receiving placebo. Other laboratory abnormalities (hemoglobin, WBCs, alkaline phosphatase, and serum creatinine) occurred with similar frequencies in the two groups.

**Post-market adverse drug reactions:** The following events have been reported voluntarily during post-approval use of Valacyclovir in clinical practice. These events have been chosen for inclusion due to either their seriousness, frequency of reporting, causal connection to Valacyclovir, or a combination of these factors. Post-market AEs are reported spontaneously from a population of unknown size, thus estimates of frequency cannot be made. Allergic: Acute hypersensitivity reactions including anaphylaxis, angioedema, dyspnea, pruritus, rash, and urticaria. Central Nervous System Symptoms: Headache. Reports of neurological reactions including dizziness, confusion, hallucinations (auditory and visual), aggressive behavior, decreased consciousness, tremor, ataxia, dysarthria, convulsions, encephalopathy, coma, mania, and seizures. Agitation and psychotic symptoms have also been reported. These events are generally reversible and usually seen in patients with renal impairment or other predisposing factors. General: Facial edema, hypertension, and tachycardia. Gastrointestinal: Nausea, abdominal discomfort, vomiting, and diarrhea. Hematological: Reports of thrombocytopenia, aplastic anemia, leukocytoclastic vasculitis, and TTP/HUS. Leukopenia has mainly been reported in immunocompromised patients. Hepatobiliary Tract and Pancreas: Reports of reversible increases in liver function test and hepatitis. Ophthalmologic: Visual abnormalities. Renal: Reports of renal impairment, elevated blood creatinine and BUN, acute renal failure, renal pain, and hematuria. Renal pain may be associated with renal failure. Skin: Erythema multiforme and rashes including photosensitivity. Other: There have been reports of renal insufficiency, microangiopathic hemolytic anemia, and thrombocytopenia (sometimes in combination) in severely immunocompromised patients, particularly those with advanced HIV disease, receiving high doses (8000 mg QD) of Valacyclovir for prolonged periods in clinical trials. These findings have also been observed in patients not treated with Valacyclovir who have the same underlying or concurrent conditions.

**Drug interactions:**

No clinically significant interactions have been identified. No dosage adjustment is recommended when Valacyclovir is coadministered with digoxin, antacids, thiazide diuretics, cimetidine, or probenecid in subjects with normal renal function.

Cimetidine and probenecid increase the area under the curve (AUC) of acyclovir and reduce acyclovir renal clearance after Valacyclovir administration (1000 mg). However, no dosage adjustment is necessary at this dose because of the wide therapeutic index of acyclovir.

Drug-Food Interactions: There is no known interaction with food.

Drug-Herb Interactions: Interactions with herbal products have not been established.
Drug-Laboratory Test Interactions: Interactions with laboratory tests have not been established.

**Dosage and administration:**

The recommended dose for the ENSIGN trial is 2 g orally t.i.d. for 14 days (day 1 to day 15). This dose has been calculated to give a similar AUC as 10 mg/kg of intravenous acyclovir administered every 8 hours. This is the same dose regimen used in a previous phase I clinical trial of ADV/HSV-tk plus acyclovir and topotecan in patients with recurrent ovarian cancer.[81] NOTE: For patients with serum creatinine level between 1.6−2.0 × ULN, valacyclovir dose will be reduced 50% (i.e., 1 g t.i.d.).

Recommended Dose and Dosage Adjustment

Patients who develop signs of TTP/HUS or neurotoxicity (e.g., encephalopathy, seizures) will be removed from treatment.

**Patients with acute or chronic renal impairment:** Caution is advised when administering Valacyclovir to patients with impaired renal function. Adequate hydration should be maintained.

Pharmacokinetic and safety evaluations following administration of oral valacyclovir hydrochloride have been performed in patients with renal impairment and volunteers with end-stage renal disease managed by hemodialysis. Valacyclovir dosing in patients with renal impairment is shown in Table 4.

*Table 4. Valacyclovir dosing in patients with renal impairment*

| | **Creatinine Clearance (mL/min)** | | | |
|---|---|---|---|---|
| | **> 50** | **30 to <50** | **10 to <30** | **< 10** |
| **Herpes Zoster** | 1000 mg every 8 hours† | 1000 mg every 12 hours | 1000 mg every 24 hours | 500 mg every 24 hours |
| **Recurrent Episodes of Genital Herpes** | 500 mg every 12 hours† | 500 mg every 12 hours† | 500 mg every 24 hours | 500 mg every 24 hours |
| Suppression of genital herpes for immunocompetent patients | 1000 mg every 24 hours† | 1000 mg every 24 hours† | 500 mg every 24 hours | 500 mg every 24 hours |
| Alternate dose for immunocompetent patients with less than or equal to 9 recurrences/year | 500 mg every 24 hours† | 500 mg every 24 hours† | 500 mg every 48 hours | 500 mg every 48 hours |
| HIV-infected patients | 500 mg every 12 hours† | 500 mg every 12 hours† | 500 mg every 24 hours | 500 mg every 24 hours |
| **Initial Episode of Genital Herpes** | 1000 mg every 12 hours† | 1000 mg every 12 hours† | 1000 mg every 24 hours | 500 mg every 24 hours |
| **Cold Sores (Herpes Labialis)§** | Two 2000 mg doses taken 12 hours apart† | Two 1000 mg doses taken 12 hours apart | Two 500 mg doses taken 12 hours apart | 500 mg single dose |

†Standard dose - adjustment not necessary
§Do not exceed one day of treatment.

**Overdosage:**

Activated charcoal may be administered to aid in the removal of unabsorbed drug. General supportive measures are recommended. Acute renal failure and neurological symptoms, including confusion, hallucinations, agitation, decreased consciousness and coma, and nausea and vomiting may also occur. Caution is required to prevent inadvertent overdose. Many of the reported cases involved renally impaired and geriatric patients receiving repeated overdoses due to lack of appropriate dosage reduction.

**Drug interactions:**

Cimetidine and Probenecid: Coadministration of probenecid with IV acyclovir has been shown to increase the mean elimination half-life and AUC of acyclovir, leading to corresponding reductions in acyclovir urinary excretion and renal clearance. The administration of cimetidine and probenecid, alone or in combination, reduced the rate

but not the extent of Valacyclovir conversion to acyclovir. Reductions in the renal clearance of acyclovir were observed, resulting in higher acyclovir plasma concentrations. In volunteers with normal renal function, the renal clearance of acyclovir was reduced by approximately 22% and 33% with concomitant cimetidine and probenecid administration, respectively. Renal clearance of acyclovir was reduced by approximately 46% in patients receiving cimetidine, probenecid, and valacyclovir hydrochloride.
An additive increase in acyclovir AUC with concomitant administration of valacyclovir hydrochloride, cimetidine, and probenecid has also been observed. Acyclovir Cmax was increased 8.4% ± 27.8%, 22.5% ± 25.3%, and 29.6% ± 27.5% by cimetidine, probenecid, and combination treatment (concomitant cimetidine and probenecid administration), respectively. Acyclovir AUC (0 to 24 hours) was increased 31.9% ± 22.9%, 49.0% ± 27.9%, and 77.9% ± 38.6% by cimetidine, probenecid, and combination treatment, respectively. Digoxin: The pharmacokinetics of digoxin (two 0.75 mg doses, 12 hours apart) were not affected by multiple-dose administration of Valacyclovir (1000 mg every 8 hours for 8 days beginning 12 hours before digoxin dosing) in a study with 12 volunteers. Acyclovir pharmacokinetics after single-dose administration of Valacyclovir (1000 mg) remained unchanged when the same dose was administered immediately after the second of two 0.75 mg doses of digoxin given 12 hours apart. Antacids: The administration of an aluminum hydroxide and magnesium hydroxide-containing antacid either 30 minutes before or 65 minutes after administration of 1000 mg Valacyclovir had no effect on the pharmacokinetics of acyclovir in a study with 18 volunteers. Thiazide diuretics: Thiazide diuretics did not affect acyclovir pharmacokinetics after administration of valacyclovir hydrochloride in a geriatric population.
**Storage and stability:**
Valacyclovir should be stored between 15° and 30°C and protected from light.
Dosage forms, composition, and packaging: Valacyclovir 500 mg caplets are blue, film-coated, capsule-shaped tablets printed with "Valacyclovir 500 mg". Each blue caplet contains valacyclovir hydrochloride equivalent to 500 mg Valacyclovir and the inactive ingredients carnauba wax, cellulose, crospovidone, hydroxypropyl methylcellulose, Indigotine Aluminum Lake, magnesium stearate, polyethylene glycol, polysorbate 80, povidone, silicon dioxide, and titanium dioxide. The blue, film-coated caplets are printed with edible white ink. Valacyclovir 1000 mg caplets are white, film-coated, capsule-shaped tablets engraved with "GX CF2". Each white caplet contains valacyclovir hydrochloride equivalent to 1000 mg valacyclovir and the inactive ingredients carnauba wax, cellulose, crospovidone, hydroxypropyl methylcellulose, magnesium stearate, polyethylene glycol, polysorbate 80, povidone, silicon dioxide, and titanium dioxide. The white, film-coated caplets are engraved. The 500 mg and 1000 mg caplets are available in blister packs of 30 and 21 caplets, respectively.

### 9.3 SBRT

RT is traditionally used for its direct cytocidal effect on cancer cells; however, it also has immunomodulatory properties that can be harnessed to potentiate an immune response.[92,93] Ionizing radiation causes immunogenic cancer cell death, modulates antigen presentation by cancer cells, and most importantly alters the microenvironment within the irradiated field.[94-97] Lymphocytes are exquisitely sensitive to ionizing radiation, and the direct effect of RT on TILs is generally cytocidal.[98] This results in temporary selective ablation of immune cells within the irradiated target, depleting CTLs and NK cells directed against the tumor as well as Tregs that suppress local antitumor immunity. The relative importance of the effect of RT on these populations remains unclear, but it is evident that the damaging effects of this physical insult are sensed by the immune system, with systemic implications.
Radiation-induced immunogenic cell death is characterized by the release of tumor antigens in the context of endogenous adjuvants that facilitate the priming of antitumor CTLs.[99] Important components of immunogenic cell death include translocation of calreticulin (CRT) to the tumor cell membrane and release of ATP and other endogenous adjuvants such as high mobility group box 1 (HMGB1),[100] uric acid,[101] and heat-shock proteins (HSPs).[102,103] These endogenous adjuvants act through the toll-like receptors (TLRs) to facilitate DC maturation.[104-106] TLRs in the mammalian immune system were first described as pattern recognition receptors

that respond to pathogen-associated molecular patterns (PAMPs) such as endotoxin from bacteria and double-stranded RNA from viruses.[107] However, growing evidence indicates that TLRs have a broader function by mediating the response to danger-associated molecular patterns (DAMPs).[108] DAMPs are a larger class of molecules including PAMPs as well as endogenous, evolutionarily conserved intracellular molecules that are released upon necrotic cell death. By linking the innate and adaptive immune system through activation of antigen-presenting cells, release of DAMPs is a key aspect of RT-mediated immunogenic cell death.

Another key component of the proimmunogenic effect of RT is the facilitation of tumor antigen uptake by DCs and their cross-presentation on MHC class I molecules.[109] In fact, radiation induces MHC class I molecules in both tumors and normal tissue.[110, 111] By enhancing the presentation of antigens released by its cytocidal effect, RT potentiates cross-priming of tumor-specific CTLs in the lymph nodes. Exogenous antigens can access the cross-presentation pathway by a variety of means, but the most important for antitumor immunity is the uptake of cell-associated antigens mediated by CRT translocation from the endoplasmic reticulum of tumor cells to the cell surface. Ionizing radiation causes CRT to translocate to the tumor cell surface where it acts as an “eat me” signal to macrophages and DCs, which internalize CRT-expressing tumor cells.[112] This process is mediated by the common HSP receptor CD91 and is a necessary part of anthracycline and radiation-induced immunogenic cell death.[113-115] Radiation induces the translocation of CRT on the tumor cell surface and the release of DAMPs, HMGB1, and ATP. These signals have been shown to be necessary and sufficient in a model of radiation-induced antitumor immunity.[116, 117]

There is evidence from both human beings and mice that tumor-associated antigens are cross-presented by DCs after RT, and this results in the cross-priming of tumor-specific CTLs. By experimental necessity, much of this evidence comes from murine tumor lines transfected to express model antigens, which allow for the measurement of specific CTL responses against known peptide epitopes. A single fraction of 20-Gy ionizing radiation results in cross-presentation of an epitope from the SIY model antigen in an *in vivo* melanoma model.[118] In a different melanoma model, both single-dose (15 Gy) and fractionated RT resulted in cross-priming of CTLs in the tumor and tumor draining lymph nodes, with fractionated treatment resulting in a smaller degree of cross-priming.[119] Other investigators have used this model to study the effect of dose and fractionation on cross-priming and have found that the number of CTLs generated correlates with radiation dose. However, fractionated RT resulted in approximately the same number of primed CTLs as single-dose RT.[120] Radiation-induced cross-priming is dependent on host TLR-4 signaling.[100] These findings are consistent with evidence from prostate cancer patients who developed prostate-specific CTLs after RT and vaccination with a poxviral vaccine encoding PSA.[121]

Immunogenic cell death alone may not be sufficient to mediate a robust antitumor immune response, because the resident DCs within tumors maintain tolerance.[122] Intratumoral injection of exogenous DCs have been used as a cancer immunotherapy, and RT has been shown to stimulate an effective antitumor CTL response among patients treated with this method.[123-126] In some experimental systems, RT overcame the suppressive effect of tumor resident DCs by recruiting new monocyte-derived DCs (mDCs) that have not been exposed to the regulatory effects of the tumor microenvironment. Tumor irradiation recruits these mDCs to tumors after treatment with a single large fraction of 25 Gy.[127] In summary, RT induces multiple intracellular adhesion molecules, chemokines, and cytokines that mediate naïve DC recruitment and may, at least in part, subvert the immune-tolerant microenvironment characteristic of established tumors.[128-130]

RT facilitates the recruitment of effector T-cells to tumors through chemokine induction. Chemokines are known to be important for the recruitment of leukocytes to tumors as part of antitumor immunity.[131,132] However, tumors with their immunosuppressive milieu tend to produce chemokines that recruit Tregs and other suppressive elements.[133,134] Without effective chemotaxis, lymphocytes primed against tumor antigens cannot home to tumors and carry out their effector function. Chemokine ligand 16 (CXCL16) has been identified as a prognostic factor in colorectal and renal cell cancers and correlates with improved survival and increased TILs.[135-137] In the 4T1 mouse breast cancer model, radiation induces the production of CXCL16, which mediates T cell recruitment to tumors by binding to chemokine receptor 6 on T cells.[138] Radiation also has effects on the tumor vascular endothelium, inducing cell adhesion molecules that further promote recruitment of antitumor CTLs.[139] Although it does not

explain the systemic immune effects of radiation, chemotaxis may partially account for the direct effects of RT on tumor control.

The positive effect of ionizing radiation on antitumor immune response supports the hypothesis that the immune system is responsible for the abscopal effect of RT. Originally described by Mole, the abscopal (from the Latin ab and the Greek Scopus, away from the target) effect of RT is a phenomenon by which a primary tumor is irradiated and a response is seen at distant metastatic sites outside of the path of the radiation.[140-146] Even the "in field" effects of radiation have been shown to be dependent on the immune system. CD8+ T cells and type I IFN are required for RT-induced tumor regression, as their depletion revokes RT-mediated tumor control.[118, 127,147-152]

Despite the observation that radiation induces effects sensed by the immune system and modulates the antitumor immune response, abscopal responses are rarely seen in clinical practice. Although evidence suggests that RT alone is sufficient to provide the necessary signals for cross-priming of CTLs against tumor antigens, this adjuvant effect of radiation appears to be relatively weak. However, the rare radiation-induced systemic abscopal response can be facilitated by additional immune manipulation. Although radiation primes new anti-tumor CTLs, these CTLs are usually unable to overcome the suppressive effect of the tumor microenvironment at distant untreated metastatic sites. This provides the rationale for combining systemic immunotherapies with RT.

According to traditional radiobiology, the cytotoxic effects of ionizing radiation on tumor cells are primarily due to the production of DNA double-strand breaks followed by cell death, either via apoptosis, necrosis, autophagy, mitotic catastrophe, or replicative senescence.[153] This traditional view of radiation-induced cell death is rapidly evolving to take into account the contribution of the tumor microenvironment and host antitumor immunity. This is particularly evident in the advent of immune checkpoint inhibitors to overcome the immunosuppressive environment of established tumors.[154-156] Notably, recruitment of the host's immune system as a contributor of the "in field" response to RT can result in immune memory, an advantageous systemic effect that transcends the localized nature of this treatment modality.[157]

Ionizing radiation alone may not be sufficient to produce clinically observable effects. *In vivo* and in the clinic, ionizing radiation-produced proimmunogenic effects are often masked by the overwhelming immunosuppressive microenvironment that characterizes established cancers.[158] Nonetheless, when some barriers of established immunosuppression are removed, for instance by adding immune checkpoint inhibitors (such as anti-CTLA-4, anti-PD-1, and anti-PD-L1) to local RT, the proimmunogenic effects of ionizing radiation are leveraged and foster immune-mediated tumor rejection is lifted, facilitating host anticancer immune responses. This could, at least in part, explain the enhanced local and systemic clinical benefits of combination regimens.[159-163] Evidence-based medicine has suggested that RT in combination with an immunomodulatory agent (anti-CTLA-4, PD-1, PD-L1, or New York-esophageal squamous cell carcinoma-1) can elicit immune-mediated abscopal effects in non-targeted tumors. Concurrent RT with a human monoclonal anti-CTLA-4 antibody induced immune-mediated abscopal effects in poorly immunogenic preclinical tumor models and metastatic melanoma and metastatic lung adenocarcinoma patients. This treatment combination has also been shown to increase TIL activity, induce tumor regression, and normalize tumor markers. Immunotherapeutic strategies aimed at overcoming immune tolerance and improving the activation of antitumor T cells represent a new promising therapeutic approach.[164] Among them, the human anti-CTLA-4 antibody in combination with RT still remains investigational.

Historically, abscopal responses are of rare occurrence. Few cases have been reported in several tumor types, including melanoma, renal cell carcinoma, metastatic lung adenocarcinoma, and lymphoma.[165-170] The host's immune system senses the effects that ionizing radiation provokes in irradiated tissues. This gave support to the hypothesis that the tumor could become an *in vivo* vaccine by adding an immunomodulator to ionizing RT.[171, 172] With the confirmation of this hypothesis, studies are investigating the link between the abscopal effect of RT and RT-induced antitumor response.[173-175] When combined preclinically with CTLA-4 blockade, fractionated, but not single-dose, radiation demonstrated abscopal effects in the 4T1 syngeneic murine model of mammary carcinoma[176] and in two additional preclinical cancer models.[177] Consistently, in each of the clinical cases in which abscopal effects were reported after CTLA-4 blockade and RT (see table below), the patient had received multifractionated RT targeting a visceral lesion.[178-180] The lack of benefit of ipilimumab addition to RT in a large prospective randomized trial of castration-resistant metastatic prostate cancer may be explained by the facts that

a bone lesion (instead of a visceral metastasis) was treated and that a single 8-Gy dose was used.[181] It is notable that single-dose radiation induced the three classic components of immunogenic cell death *in vitro* (CRT translocation, HMGB1 release, and ATP release) in a dose-dependent manner. *The in vivo inferiority of single-dose versus fractionated RT may reflect a mechanism dependent on the tumor microenvironment rather than on the immunogenicity of cancer cells.*
The abscopal cases reported (see table below) and many emerging systemic responses in the setting of CTLA-4 and PD-L1 blockade have occurred with biologic doses that are certainly not ablative.

*Table 5. Abscopal responses after combination treatment with RT and CTLA-4 checkpoint inhibitor*

| Reference | Radiation Regimen | CTLA-4 Antibody Dose | Tumor Type | Target | Setting |
|---|---|---|---|---|---|
| Dewan[168] | 6 Gy x 5 f<br>8 Gy x 3 f | 10 mg/kg | Breast cancer | Primary tumor | Preclinical |
| Hinikerfl[169] | 18 Gy x 3 f | 3 mg/kg | Melanoma | Liver metastasis | Clinical |
| Postow[170] | 9.5 Gy x 3 f | 10 mg/kg | Melanoma | Paraspinal metastasis | Clinical |
| Golden[171] | 6 Gy x 5 f | 3 mg/kg | Lung cancer | Liver metastasis | Clinical |
| CTLA-4 = cytotoxic T lymphocyte-associated protein-4; f = fraction. | | | | | |

In the above clinical studies, patients that received immunotherapy plus ionizing RT were successfully immunized against their tumor, both locally and systemically. The standing question is whether more patients would have responded if their irradiated metastatic lesion had been ablated or if ablative doses of stereotactic radiosurgery had been applied to all clinically and radiologically detectable metastatic sites.

**Dosage guidelines:**
SBRT of 30 Gy (6 Gy X 5 fractions) will be administered over 2 weeks starting from day 2 to day 14 of the study.
**Delivery:**
Tumor target will be decided by PET/CT scan. For the planned dosing regimen, SBRT simulation will be done before treatment. For subjects with multiple tumors, the largest tumor will be selected. Sedation is not necessary for SBRT.
**Side effects:**
Side effects of the proposed low-dose SBRT will be minimal. Potential side effects include fatigue, skin irritation, esophagitis, pneumonitis, bronchitis, rib fracture, chest wall pain, and spinal cord injury.

**9.4 Nivolumab**
**Common trade name(s):** Opdivo®
**Classification:** monoclonal antibody
**Mechanism of action:**
Nivolumab is a fully human immune checkpoint inhibitor monoclonal antibody. Nivolumab enhances antitumor immunity by selectively blocking the interaction of PD-1 with its known ligands, PD-L1 and PD-L2, disrupting the negative regulation of T cell activation and proliferation.[182, 183]
**Uses:**
Primary uses: Melanoma, [184] Lung cancer, Squamous and non-squamous NSCLC [185]
Other uses: Renal cell cancer, [186,187] Hodgkin's lymphoma[188]
**Special precautions:**
Pregnancy: In animal reproduction studies, nivolumab administration resulted in increased abortion and premature infant death. There is no available human data; however, a central function of the PD-1/PD-L1 pathway is to preserve pregnancy by maintaining maternal immune tolerance to the fetus. Therefore, based on its

mechanism of action, nivolumab may cause fetal harm if administered to a pregnant woman. Also, human IgG4 is known to cross the placental barrier. As nivolumab is an IgG4 antibody, it has the potential to be transmitted from the mother to the developing fetus where it may increase the risk of immune-mediated disorders. WOCBP are advised to use effective contraception during treatment and for five months following the last dose of nivolumab.

**Side effects:**

The table below includes AEs that presented during drug treatment but may not necessarily have a causal relationship with the drug. Because clinical trials are conducted under very specific conditions, the AE rates observed may not reflect the rates observed in clinical practice. AEs reported in more than 1% of patients in the product monograph or pivotal trials are included.

*Table 6. Nivolumab side effects*

| ORGAN SITE | SIDE EFFECT |
|---|---|
| Gastrointestinal | Emetogenic potential: low, Abdominal pain (16%, severe 2%), Constipation (24%), Nausea (29%, severe 2%), Vomiting (19%, severe 1%). |
| General disorders and administration site conditions | Extravasation hazard: none, Asthenia (19%, severe 2%), Chest discomfort, non-cardiac chest pain (13%), Edema (17%, severe 2%), Fatigue (50%, severe 7%), Peripheral edema (10%), Pyrexia (17%). |
| Immune system (see paragraph following Side Effects table) | Colitis (1-2%), Diarrhea (18-21%, severe 1-3%), Hepatitis (1%), Hyperthyroidism (2-3%), Hypothyroidism (4-8%), Nephritis, renal dysfunction (1%), Pneumonitis (1-6%). |
| Infections and infestations | Upper respiratory tract infection (11%) |
| Investigations | Alkaline phosphatase increase (14-22%), ALT increase (12-16%), AST increase (16-28%), Total bilirubin increase (3-19%), Creatinine increase (13-22%), Weight decrease (13%, severe 1%). |
| Metabolism and nutrition | Decreased appetite (35%, severe 3%). |
| Musculoskeletal and connective tissue | Arthralgia (13%), Musculoskeletal pain (36%, severe 6%). |
| Respiratory, thoracic, and mediastinal | Cough (17%). |
| Skin and subcutaneous tissue | Pruritus (19%), Rash (21%, severe <1%). |

*Clinically significant immune-mediated adverse reactions can occur, including pneumonitis, colitis, hepatitis, nephritis, hypothyroidism, and hyperthyroidism. Immune reactions may be delayed or occur after nivolumab discontinuation*. Based on the type and severity of the reaction, management may include withholding or discontinuing nivolumab, administering HD corticosteroids, and if appropriate, initiating hormone replacement therapy. Following resolution of the reaction to grade 1 or less, corticosteroids should be tapered over at least one month. Nivolumab should not be resumed while the patient is receiving immunosuppressive doses of corticosteroids or other immunosuppressive therapy. Restarting nivolumab may be considered following completion of corticosteroid taper. Permanent discontinuation of nivolumab is usually recommended following grade 3 or 4 immune-mediated reactions.

**Supply and storage:**

Injection: Bristol-Myers Squibb Company supplies nivolumab as 40 mg and 100 mg ready-to-use, single-use (preservative-free) vials in a concentration of 10 mg/mL. Refrigerate and store in original packaging to protect from light. Do not freeze or shake.[189]

**Solution and compatibility:**

• Nivolumab should be clear and colorless to pale-yellow in color. Discard if cloudy or discolored.

• Nivolumab can be infused undiluted (10 mg/mL) or diluted with normal saline or 5% dextrose in water (to 0.35 mg/mL or greater).

**Parenteral administration:**

Intermittent infusion over 60 minutes.

**Dosage guidelines:**

The ENSIGN protocol's guidelines for dosing also include consideration of ANC. Dosage may be reduced, delayed, or discontinued in patients with bone marrow depression due to cytotoxic therapy/RT or other toxicities. (See Section 6 for management of immune-mediated complications of nivolumab)

*Table 7. Nivolumab adult dosing*

| Route | Cycle Length: | Dose |
|---|---|---|
| IV | 4 weeks | 480 mg IV for one dose on day 1 (Total dose per cycle, 480 mg) |

Table 7

## 10. Prohibited concomitant therapy

**Antineoplastic therapies**

Treatment with other systemic/local anticancer agents (chemotherapy, immunotherapy, RT, hormone therapy, targeted or biologic agents) other than the protocol treatment is not permitted until disease progression is documented per modified irRC. Concomitant therapies will be continuously monitored from the first day of treatment.

## 11. Visit schedule and assessments

| Visit | Screening[a] | Baseline[b] | V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 | V9 | V10* | EOT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | **-28 to -1** | **-7 to -1** | **0±5** | **2±5** | **4±5** | **7±5** | **9±5** | **11±5** | **17±5** | **45±5** | **73±5** | **101*± 5** | |
| Informed Consent | X | | | | | | | | | | | | |
| Inclusion/Exclusion | X | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | |
| Medical History | X | | | | | | | | | | | | |
| Physical Exam[c] | X | | X | | | | | | X | X | X | X | X |
| Height | X | | | | | | | | | | | | |
| Weight | X | | X | | | | | | X | X | X | X | X |
| ECOG PS | X | | X | | | | | | X | X | X | X | X |
| 12-Lead ECG and MUGA Scan or Echocardiogram[d] | X | | | | | | | | | | | | X |
| Concomitant Therapies | | | **Continuous from the first day of treatment** | | | | | | | | | | |
| Hematology[e] | X | | X | | | | | | X | X | X | X | X |
| Clinical Chemistry[e] | X | | X | | | | | | X | X | X | X | X |
| PT/INR/aPTT[e] | X | | | | | | | | X | X | X | X | X |
| Serum Pregnancy (β-hCG )[f] | X | | | | | | | | | | | | |
| Brain MRI[g] | X | | | | | | | | | | | | |
| Tumor Assessments (RECIST 1:1 and irRC)[h] | X | | | | | | | | | | | | X |
| Biopsy[i] | | | X | | | | | | X | | | | |
| Correlative Studies Blood Collection[j] | X | | | | | | | | X | | X | | |
| Adverse Events and Serious Adverse Events | | | **From informed consent signing up to and including 30 days after the last treatment dose[l]** | | | | | | | | | | |
| ADV/HSV-tk Administration[k] | | | X | | | | | | | | | | |
| Valacyclovir Administration[k] | | | X | | | | | | | | | | |
| SBRT Administration[k] | | | | X | X | X | X | X | | | | | |
| Nivolumab Administration[k] | | | | | | | | | X | X | X | X | |

Abbreviations: aPTT = activated partial thromboplastin time; AE = adverse event; ADV/HSV-tk = adenovirus-mediated expression of herpes simplex virus thymidine kinase; ANC = absolute neutrophil count; β-hCG = beta-human chorionic gonadotropin; CBC = complete blood count; CT = computed tomography; ECG = electrocardiogram; ECOG PS = Eastern Cooperative Oncology Group performance status; EOT = end of treatment; INR = international normalized ratio; irRC = immune-related response criteria; MRI – magnetic resonance imaging; MUGA, multigated acquisition; PET = positron emission tomography; PT = prothrombin time; RECIST = Response Evaluation Criteria in Solid Tumors; SAE = serious adverse event; SBRT = stereotactic body radiation therapy; WBC = white blood cell. A window of ± 5 days is allowed for study visits and assessments (except as otherwise specified).

[a]Within 28 days prior to ADV/HSV-tk administration on day 0.

[b]Within 7 days prior to ADV/HSV-tk administration on day 0. Only screening procedures not performed within 7 days of ADV/HSV-tk dosing are required at baseline.

[c]The baseline symptom-medical history and physical examination are not required if the screening medical history and physical examination were conducted within 7 to 28 days prior to day 0 ADV/HSV-tk dosing.

Vital sign (blood pressure, heart rate, and oral temperature) measurements will be performed at each physical exam.

[d]A 12-lead ECG will be performed at screening, at EOT, and when clinically indicated. MUGA scan or echocardiogram will be performed when clinically indicated. The same method (echocardiogram or MUGA scan) must be used throughout the duration of the study.

[e]A blood sample for CBC with platelet count and differential WBC count will be obtained at screening, on days 0 and 17, every 4 weeks thereafter until completion of the protocol-specified treatment , at EOT, and when clinically indicated.

If a patient is found to have an ANC <1.5 x 10^9/L, platelet count < 100 x 10^9/L, or both, the CBC with differential should be repeated at least every other day until the ANC and platelet count have exceeded these values.

[e]A blood sample for clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, aspartate transaminase, and alanine transaminase) and evaluation of magnesium and lactate dehydrogenase will be obtained at screening, on days 0 and 17, every 4 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated.

[e]PT/INR and aPTT will be tested at screening, before every cycle of nivolumab, and at EOT.

[f]For WOCBP, the results of a serum β-hCG pregnancy test must be negative within 7 days before study treatment start. If the screening serum β-hCG pregnancy test is performed more than 7 days before ADV/HSV-tk dosing, it must be repeated at baseline, with results known to be negative prior to the first treatment dose. The test is to be repeated as clinically indicated.

[g] Brain MRI will be performed at screening.

[h]Relapsed/refractory and/or metastatic patients will be evaluated with /CT scan of the thorax, abdomen, and pelvis at screening, every eight (8) weeks thereafter until completion of the protocol-specified treatment and/or at the discretion of the treating physician, at EOT, and when clinically indicated. For patients with equivocal CT scan results, PET scan will be performed.

[i]In patients with accessible tumor, biopsies will be conducted at Visit 1, after the first dose of nivolumab (± 2 days), and if the patient progresses while on treatment, before the patient starts the new treatment.

Banked tumor tissue obtained as part of the patient's standard care and additional biopsies will be evaluated at a later time for assessment of Th1 response (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF) and TILs before and after ADV/HSV-tk + Valacyclovir therapy.

[j]Blood samples for correlative studies will be collected at screening, after the first dose of nivolumab (± 2 days), and at the end of Cycle 2 of nivolumab. An additional sample will be collected from patients whose disease progresses while on treatment. Blood samples will be collected into standard vacutainer tubes (3 green top tubes). Samples will be evaluated for profile of circulating suppressor and effector immunocytes and cytokines.

[k]ADV/HSV-tk will be injected intratumorally at 5 x $10^{11}$ viral particles in a 2-mL total volume on day 0 of the study. The study coordinator will dispense the 14-day quantity of Valacyclovir on Day 0 of the study. The patient will be instructed to take Valacyclovir (2 g) orally t.i.d. for 14 days (day 1 to day 15 of the study). NOTE: For patients with serum creatinine level between 1.6−2.0 × ULN, valacyclovir dose will be reduced 50% (i.e., 1 g t.i.d.).

SBRT of 30 Gy (6 Gy X 5 fractions) will be administered over 2 weeks from day 2 to day 14 of the study. Nivolumab (480 mg) will be administered intravenously over 30 minutes every 4 weeks (± 1 day) starting on day 17 (± 1 day) of the study and continuing until disease progression, unacceptable toxicity, or up to 12 months in patients without disease progression.

[l]AEs and SAEs will be captured from the time of informed consent signing up to and including 30 days after the final dose of nivolumab. Study treatment-related SAEs occurring beyond 30 days after the last dose of nivolumab and any study patient death should also be reported.

*Patients will be treated until disease progression, unacceptable toxicity, or up to 12 months in patients without disease progression.

**12. Assessment types**

A window of ± 5 days is allowed for study visits and assessments (except as otherwise specified). If extenuating circumstances prevent a patient from beginning treatment or completing a scheduled assessment within this time frame, the patient may continue in the study only with written permission of the medical monitor. Additional schedules that specify study days on which these assessments are to be performed will be provided in the study manual as needed.

Screening: Within 28 days prior to ADV/HSV-tk administration on day 0.

Baseline: Within 1 to 7 days prior to ADV/HSV-tk administration on day 0.

Only screening procedures not performed within 7 days of ADV/HSV-tk dosing are required at baseline.

A screening medical history and physical exam will be performed. Patients presenting with any medical history, physical exam, or laboratory abnormality that, in the opinion of the treating physician, would put their safety at risk will be excluded. A physical exam will be performed on day 0 and 17; every 4 weeks thereafter until completion of the protocol-specified treatment; and at EOT. Vital sign (blood pressure, heart rate, and oral temperature) measurements will be performed at each physical exam. A 12-lead ECG will be performed at screening, at EOT, and when clinically indicated. MUGA scan or ECHO will be performed when clinically indicated. The same method (ECHO or MUGA scan) must be used throughout the duration of the study. A blood sample for CBC with platelet count and differential WBC count will be obtained at screening, on days 0 and 17, every 4 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. If a patient is found to have an ANC <1.5 x 10^9/L, platelet count < 100 x 10^9/L, or both, the CBC with differential should be repeated at least every other day until the ANC and platelet count have exceeded these values. A blood sample for clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, chloride, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, and ALT) and evaluation of magnesium and lactate dehydrogenase will be obtained at screening, on days 0 and 17, every 4 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. PT, INR, aPTT testing will be performed at screening, on day 17, every 4 weeks thereafter until completion of the protocol-specified treatment, and at EOT. For WOCBP, the results of a serum β-hCG pregnancy test must be negative within 7 days before the first treatment dose is administered. If the screening serum β-hCG pregnancy test is performed more than 7 days before ADV/HSV-tk dosing, it must be repeated at baseline, with results known to be negative prior to first treatment dose. β-hCG pregnancy testing is to be repeated as clinically indicated. Laboratory tests may be done more frequently if medically indicated. Participants will be assessed for AEs and SAEs from informed consent signing up to and including 30 days after the last nivolumab dose. Study treatment-related AEs occurring beyond 30 days from the last dose of nivolumab and any study patient death should also be reported. Toxicities will be defined as any treatment-related death or any ≥ Grade 3 toxicity excluding alopecia and constitutional symptoms as assessed by NCI CTCAE v4.03. Patients whose treatment is interrupted or permanently discontinued due to an AE including abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4-week intervals until resolution or stabilization of the event, whichever comes first. The maximum time allowed for toxicity-related treatment interruption is 21 days from the intended dosing day. If interruption is > 3 weeks, the patient must be discontinued from the study treatment. However, the patient will continue to be followed for toxicity. Brain MRI will be performed at screening. Relapsed/refractory and/or metastatic patients will be evaluated with CT scan of the thorax, abdomen, and pelvis at screening, every eight (8) weeks thereafter until completion of the protocol-specified treatment and/or at the discretion of the treating physician, at EOT, and as clinically indicated. For patients with equivocal CT scan results, PET scan will be performed. In patients with accessible tumor, biopsies will be conducted at day 0, after the first dose of nivolumab (± 2 days), and if the patient

progresses while on treatment, before the patient starts the new treatment.
Banked tumor tissue obtained as part of the patient's standard care and additional biopsies will be evaluated at later time for assessment of Th1 response (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF) and TILs before and after ADV/HSV-tk + Valacyclovir therapy. The biopsy procedures will be either a CT-guided biopsy for peripheral lesions or an endobronchial ultrasound for central lesions or adenopathy. Biopsy will be performed at day 0 and after the first dose of nivolumab. Blood samples for correlative studies will be collected at screening, after the first dose (± 2 days) of nivolumab, and at the end of Cycle 2 of nivolumab. An additional sample will be collected from subjects whose disease progresses while on treatment. Blood samples will be collected into standard vacutainer tubes (3 green top tubes). Samples will be evaluated for profile of circulating suppressor and effector immunocytes and cytokines.

ADV/HSV-tk ($5 \times 10^{11}$ viral particles) in a 2-mL total volume will be injected intratumorally on day 0 of the study. Valacyclovir will be administered 24 hours after the gene vector injection at a dose of 2 g orally t.i.d. for 14 days. NOTE: For patients with serum creatinine level between 1.6−2.0 × ULN, valacyclovir dose will be reduced 50% (i.e., 1 g t.i.d.). Valacyclovir treatment will be administered from day 1 to day 15 of the study. SBRT of 30 Gy (6 Gy X 5 fractions) will be administered over 2 weeks from day 2 to day 14 of the study. Nivolumab (480 mg) will be intravenously administered over 30 minutes every 4 weeks (± 1 day) starting on day 17 (± 1 day) of the study and continuing until disease progression, unacceptable toxicity, or up to 12 months in patients without disease progression.

### 12.1 Criteria for response

The main endpoint in this study is the ORR of ADV/HSV-tk + Valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before nivolumab in patients with metastatic squamous or non-squamous NSCLC and metastatic uveal melanoma. RECIST criteria 1.1 will be used to select patients with detectable or measurable disease as part of the initial inclusion process. Modified irRC and RECIST 1.1 will be used to assess treatment response.

### 12.2 RECIST

RECIST offers a simplified, conservative, extraction of imaging data for wide application in clinical trials. They presume that linear measures are an adequate substitute for two-dimensional methods and register four response categories.[190]

**Target Lesions (Main Tumor)**

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, using the baseline sum diameters as the reference.
- Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, using the smallest sum on study (includes the baseline sum) as the reference. In addition to the relative 20% increase, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression)
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, using the smallest sum diameters while on study as the reference.

**Non-Target Lesions (Lymph Nodes)**

- CR: Disappearance of all non-target lesions and normalization of tumor marker levels. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker levels above the normal limits.
- PPD: Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.
- *Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or principal investigator).*

### 12.3 Modified irRC, derived from RECIST 1.1[191]

This new classification is based on recent clinical studies showing delays in tumor regression or stabilization with cancer immunotherapies. For the **ENSIGN** trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC, which uses unidimensional measurements. For modified irRC, only target and measurable lesions are taken into account. In contrast to RECIST 1.1, the modified irRC criteria (a) require confirmation of both progression and response by imaging at eight 8 weeks after initial imaging (evidence of cancer progression within the first 3 months on bone scan only should be interpreted with extreme caution due to risk of tumor flare) and (b) do not necessarily score the appearance of new lesions as PD if the sum of the diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%. The same assessment method and technique should be used to characterize each identified and reported target lesion(s) at baseline, during the trial, and at EOT. All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are displayed below. Modified irRC are defined as follows:

- New measurable lesions: Incorporated into tumor burden.
- New non-measurable lesions: Do not define progression but preclude irCR.
- Overall irCR: Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm or less.
- Overall irPR: ≥ 30% decrease in the sum of the longest diameters of target and new measurable lesions.
- Overall irSD: Neither sufficient shrinkage to qualify for irCR or irPR (compared to baseline) nor sufficient increase to qualify for irPD (compared to nadir), using the sum of the longest diameters of target and new measurable lesions.
- Overall irPD: ≥ 20% increase in the sum of the longest diameters of target and new measurable lesions increases (compared to nadir), confirmed by a repeat, consecutive observation at least 6 weeks (normally it should be done at 8 weeks) from the date first documented.

*Documentation of immune-related PD (based on modified irRC) does not mandate discontinuation of the study treatment even after irPD is confirmed with CT scan 6 weeks after the initial observation of irPD.*

### 12.4 Withdrawal of subjects from study

**Subjects must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Therapy will be discontinued temporarily or permanently for cytokine release syndrome.
- Therapy will be discontinued temporarily or permanently for elevated ALT (> 5 x ULN).
- Any Grade 4 or greater AE.

- Severe (Grade 3) or life-threatening (Grade 4) immune-mediated complications.
- TTP/HUS.
- Neurotoxicity (e.g., encephalopathy, seizures).
- Dose interruption that exceeds 21 days.
- Subject withdraws consent from study treatment and study procedures. A subject must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Subject is lost to follow-up.
- Death.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable. In all cases, the reason for withdrawal must be recorded in the CRF and the subject's medical records.

### 12.5 Pregnancy

WOCBP must have a negative serum pregnancy test within 7 days of the administration of the first study treatment. Pregnancy testing is required at screening or whenever pregnancy is suspected.

WOCBP, defined as all women physiologically capable of becoming pregnant, must use highly effective contraception during the study and for 5 months after stopping nivolumab. Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception)
- Sterilization: Surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before study treatment start. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow-up hormone level assessment.
- Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). (For female subjects on the study, the vasectomized male partner should be the sole partner for that subject)
- Use of a combination of both of the following:
    - Placement of an intrauterine device (IUD) or intrauterine system (IUS)
    - Barrier methods of contraception: Condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

Sexually active males must use a condom during intercourse while enrolled in the study and for 5 months after stopping treatment and should not father a child in this period. A condom is required to be used also by vasectomized men to prevent delivery of the drug via seminal fluid. Female partners of male patients must also be advised to use one of the following contraception methods: (1) IUD or IUS or (2) prior male/female sterilization.

## 13. Safety monitoring and reporting

Information about all AEs, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test, or other means, will be collected, recorded, and followed as appropriate.

### 13.1 AEs

#### 13.1.1 Definitions and reporting

AEs that begin or worsen after informed consent should be recorded in the AEs CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. AE monitoring should be continued up to an including 30 days following the last dose of study treatment. AEs (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

The occurrence of AEs should be sought by non-directive questioning of the patient at each study visit. AEs may also be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each AE should be evaluated to determine:

1. Severity grade (CTCAE grade 1-4)
2. Duration (Start and end dates or if continuing at the Safety Follow-up Visit)
3. Relationship to the study treatment (certain, probable, possible, not likely, not related; see Section 14.2)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown)
7. Whether it is serious, where a SAE is defined as in Section 14.

All AEs should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an AE is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome. Information on the known common side effects of the ENSIGN protocol drugs can be found in the package insert. This information should be included in the patient informed consent and should be discussed with the patient during the study as needed. AE monitoring should be continued up to an including 30 days following the last dose of study treatment.

#### 13.1.2 Laboratory test abnormalities

Laboratory abnormalities that constitute an AE in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy, or require changes in study treatment) should be recorded on the AEs CRF. Whenever possible, a diagnosis rather than a symptom should be provided (e.g., anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for AEs should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported AE, it is not necessary to separately record the lab/test result as an additional event. Laboratory abnormalities that do not meet the

definition of an AE should not be reported as AEs. A grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the SAE definition below and/or as per the investigator's discretion. A dose hold or medication for the lab abnormality may be required by the protocol and is still, by definition, an AE.

## 14. SAEs

### 14.1 Definitions

A SAE is an undesirable sign, symptom, or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication and not associated with any deterioration in condition
    - elective or preplanned treatment for a preexisting condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant (i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above)

### 14.2 Reporting

The principal investigator has the obligation to promptly report all patient deaths and related and unexpected AEs and SAEs to the local IRB and FDA. To ensure patient safety, every related SAE (as per principal investigator determination) occurring:

- after the patient has provided informed consent up to and including 30 days after the last treatment dose
- after protocol-specified procedures begin and 30 days after the patient has stopped study treatment
- after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient up to and including 30 days after the patient has stopped study treatment must be reported to the local IRB within 24 hours of learning of its occurrence; this includes serious, related, labeled (expected) and serious, related, and unlabeled (unexpected) adverse experiences. All deaths up to and including 30 days after the last treatment dose following completion of the active protocol therapy must be reported within 5 working days.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

Each reoccurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

The investigator should inform the IRB of the occurrence of any SAEs not previously documented in the Investigator Brochure or Package Insert (new occurrence) and thought to be related to the study drug. The

Investigator and the local IRB may need to issue an Investigator Notification to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected unexpected serious adverse reactions will be collected and reported to the competent authorities. The following categories and definitions of causal relationship to study drug should be considered for use in this clinical study:

- Certain: There is a known causal relationship between the study drug and the SAE. The event responds to withdrawal of study drug (dechallenge) and recurs with rechallenge when clinically feasible. (>95% certainty)
- Probable: There is reasonable causal relationship between the study drug and the SAE. The event responds to dechallenge. Rechallenge is not required. (65%–95% probability of relatedness)
- Possible: There is reasonable causal relationship between the study drug and the SAE. Dechallenge information is lacking or unclear. (35%–65% probability of relatedness)
- Not likely: There is temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the SAE. (5%–35% probability of relatedness)
- Not related: There is not a temporal relationship to study drug administration (too early or late or study drug not taken), or there is a known causal relationship between the SAE and another drug, concurrent disease, or other circumstance. (<5% chance of relatedness)

AEs classified as "serious" require expeditious handling and reporting to Houston Methodist Cancer Center and local IRB to comply with regulatory requirements. All related or unexpected AEs or SAEs of any of the study drugs must be immediately reported to the local IRB by the investigator or designee within 24 hours of becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site. Report SAEs within 24 hours via email or fax to:

**Houston Methodist Cancer Center** at:
hmccsaereports@houstonmethodist.org
**Fax Number: 713-790-5106**

The investigator must also report any suspected serious adverse reaction to the FDA (per 21 CFR312.32). The investigator must report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE, such as:
**(A)** A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure;
**(B)** One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug;
**(C)** An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.
The investigator must also notify the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the investigator's initial receipt of the information.

### 14.3 Pregnancy

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to the local IRB within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of

any birth defects, congenital abnormalities, or maternal and/or newborn complications. The newborn will be followed for at least 12 months.
Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the local IRB. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment and any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

## 15 Statistical methods

### 15.1 Sample size.

**Study Design and Sample Size Calculation:** For the NSCLC cohort, a sample size of 16 patients achieves 80.3% power to detect the difference between a null hypothesis of ORR of 3% and treatment ORR of 25% (alternative hypothesis) at a 0.05 significance level (i.e., alpha) in a one-sided Fisher's exact test. For the uveal melanoma cohort, a sample size of 9 patients achieves 80.4% power to detect the difference between a null hypothesis of ORR of 3% and treatment ORR of 30% (alternative hypothesis) at a 0.05 significance level (i.e., alpha) using a one-sided Fisher's exact test. Power calculations were performed using nQuery Advisor 7.0.
For both the NSCLC and uveal melanoma cohorts, an initial cohort of 3 patients will be followed for toxicity through the first cycle of nivolumab. If only 1 of 3 patients experience toxicity, then 3 more patients will be enrolled. If no more than 1 of 6 patients experience toxicity, then the trial will be fully opened. The staggering interval will be 5 weeks for the run-in cohort. Toxicity will be defined as any Grade 3 or greater toxicity excluding alopecia and constitutional symptoms as assessed by NCI CTCAE v4.03. Hematological toxicities will have a window of 21 days to resolve.

**Statistical Analysis:** DoR, OS, PFS, and CBR will also be analyzed. Safety profiles will be assessed through summaries of AEs, SAEs, AEs leading to treatment discontinuation, and treatment-related death. The safety analysis will report the frequency of all AEs and laboratory abnormalities as well as the frequency of dose interruptions and toxicity-related treatment discontinuation. Toxicity rates will be presented using the worst NCI CTCAE grade per patient.
**Data Management:** CRFs will be designed and utilized to capture all patient data. An electronic database will be designed to store patient CRFs. Data quality control will be performed regularly by the research coordinator/research nurse to ensure timely, accurate, and complete patient data collection. Queries will be generated and resolved prior to generation of interim and final summary reports.

## 16 Protocol amendments or changes in study conduct

Any change or addition to this protocol requires a written protocol amendment that must be reviewed by the investigator, local IRB, and FDA before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation, or the scientific quality of the study require additional approval by the IRB at each study center and the FDA. Examples of amendments requiring such approval are:

1. Increases in drug dose or duration of exposure of subjects
2. Significant changes in the study design (e.g., addition or deletion of a control group)
3. Increases in the number of invasive procedures
4. Addition or deletions of a test procedure required for safety monitoring

These requirements for approval should in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial. The IRB must be notified if immediate changes are made to the protocol by the investigator for safety reasons. Amendments affecting administrative aspects of the study do not require formal protocol amendments but will require IRB approval.

HOUSTON Methodist CANCER CENTER

## 17 Data safety monitoring

The Houston Methodist Research Institute (HMRI) and Houston Methodist Cancer Center will organize and maintain a Data and Safety Monitoring Plan (DSMP) for the purpose of protecting patients enrolled in clinical trials conducted at Houston Methodist Hospital. The DSMP will address any safety concerns that may arise from the conduct of these clinical trials or the administration of the clinical trial product (ENSIGN Trial).

### I. Confidentiality

#### A. Protection of Subject Privacy

Medical history and physical examination will be performed at baseline and at regular intervals for routine standard of care and research testing. No information will be given to anyone without permission from the subject. This statement guarantees confidentiality and identifies the subject as the owner of the information from research analysis. Confidentiality will be ensured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with a randomly generated identification code unique to the subject. The subject's medical information will be kept as confidential as possible within the limits of the law. The medical information may be given out if required by law. If information from this study is published in a medical journal or presented at scientific meetings, the subject's information will not be identified by name, picture, or any other personally identifying information. The following people and groups of people may look at and/or copy the subject's medical records to make sure that the study is being done properly and to check the quality of the data:

- The IRB responsible for protecting the rights and safety of the patients who take part in research studies at the Houston Methodist Cancer Center
- The U.S. FDA and other government agencies involved in keeping research safe for people

#### B. Database Protection

The database (REDCap) will be secured with password protection. The informatics manager will receive only coded information that is entered into the database under those identification numbers. Electronic communication with outside collaborators will involve only unidentifiable information.

#### C. Confidentiality During AE Reporting

AE reports and annual summaries will not include subject- or group-identifiable material. Each report will only include the identification code or subject number.

### II. AE Information

#### A. Definition

An AE is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental therapy being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.), or any combination of these.

A SAE is any AE that results in one or more of the following outcomes:

- Death
- A life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- An important medical event based upon appropriate medical judgment

**B. Classification of AE Severity**

AEs will be labeled according to severity, which is based on their impact on the patient. An AE will be termed "mild" if it does not have a major impact on the patient, "moderate" if it causes the patient some minor inconvenience, and "severe" if it causes a substantial disruption to the patient's well-being. Please note that a severe AE and an SAE are distinct terms. A subject could experience a severe AE that does not meet the above-listed definition of an SAE; alternatively, a subject could experience a moderate AE that meets the SAE definition.

**C. AE Attribution Scale**

AEs should also be classified on an assessment of relatedness to the study intervention. AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled definitely unrelated, definitely related, probably related, or possibly related to the study intervention.

**D. AE and SAE Reporting and Follow-up**

The principal investigator (PI) (or designated co-investigator [Co-I]) should evaluate all AEs and other problems occurring during a subject's participation in order to evaluate whether an event is a reportable event and whether the event requires reporting to the IRB or Sponsor or simply recorded in the research record according to the Sponsor protocol. These events include unanticipated problems involving risks to subjects or others (UPIRSOs), AEs, SAEs, adverse drug experiences, unexpected adverse drug experiences, protocol deviations, and noncompliance. A member of the research team may be responsible for collecting and recording information and may make an initial determination about the event but the Investigator's oversight and rationale for reporting/not reporting must be clearly documented according to the team's standard operating procedure.

This responsibility applies to all enrolled subjects from the time of documented informed consent to completion of study activities for that subject and/or when additional follow-up information is obtained including information that is obtained after the end of a subject's participation that would otherwise have been reportable (e.g., a death occurring within 30 days of study completion or the birth of a child with congenital anomalies).

External safety reports and other new information concerning safety should be reviewed and assessed in the same manner as events that occur to HMRI subjects with consideration about relatedness to the research and potential affect for this population.

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to

the Independent Monitors(s), IRB, and FDA in accordance with requirements. FDA regulations require Sponsors of multicenter studies to provide information about SAEs to all participating sites. However, there is no regulation requiring the Investigator to report all external events to the IRB.

UPIRSOs, noncompliance, and protocol deviations/violations that affect the scientific integrity of the study, subject rights, or subject welfare and safety must be promptly reported to the IRB via MORTI according to the table below. Events that do not require prompt reporting should be summarized and reported to the IRB at the time of continuing review in answer to the question about study progress. If, on review, the Investigator notes a trend in AEs or protocol deviations that would affect the study as a whole, this should be reported to the IRB as an UPIRSO.

| **HMRI IRB Reportable Events Deadlines** | |
|---|---|
| | |
| **Event** | **Deadline** |
| UPIRSO | 7 calendar days |
| UPIRSO involving a **death, or life-threatening event** of a HMRI participant, or a participant at a site that relies on the Houston Methodist Hospital MH as a Coordinating Center | 24 hours<br>May be initiated by email but must be submitted first following business day |
| Noncompliance | When aware |
| Protocol Deviation (non-emergency) | 7 working days |
| Protocol Deviation (emergency) | 5 working days |

## III. Data Quality and Safety Review Plan and Monitoring

### A. Data Quality and Management

1. **Description of Plan for Data Quality and Management:** The PI or study staff will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance.
2. **Frequency of Data Review for this Study:** Study data will be reviewed by the PI quarterly as follows:

| Data type | Frequency of review | Reviewer |
|---|---|---|
| Subject accrual (including compliance with protocol enrollment criteria) | Quarterly | PI, Independent Monitor(s) |
| Status of all enrolled subjects, as of date of reporting | Quarterly | PI, Independent Monitor(s) |

| | | |
|---|---|---|
| Adherence data regarding study visits and intervention | Quarterly | PI, Independent Monitor(s) |
| AEs and rates (including out-of-range lab values) | Quarterly | PI, Independent Monitor(s) |
| SAEs | Per occurrence | PI, Independent Monitor(s),<br>FDA (if applicable) |

**B. Subject Accrual and Compliance**

**1. Measurement and Reporting of Subject Accrual and Compliance with Inclusion/Exclusion Criteria**

Review of the rate of subject accrual and compliance with inclusion/exclusion criteria will occur monthly during the 4-month recruitment phase and then every 3 months to ensure that a sufficient number of participants are being enrolled and that they meet eligibility criteria.

**2. Measurement and Reporting of Participant Adherence to Treatment Protocol**

Data on adherence to the treatment protocol will be collected twice weekly by research staff and reviewed quarterly by the PI, the study statistician, and the safety officer. Adherence of participants will be evaluated by performing pill counts and by monitoring the appropriate case report form intake data measures at each visit.

**C. Stopping Rules**

This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping.

**D. Safety Review Plan**

Study progress and safety will be reviewed quarterly (and more frequently if needed). Progress reports, including patient recruitment, retention/attrition, and AEs will be provided to the Independent Monitor(s) following each of the quarterly reviews. An Annual Report will be compiled and will include a list and summary of AEs. In addition, the Annual Report will address (1) whether AE rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The Annual Report will be sent to the Independent Monitor(s) and will be forwarded to the IRB (if applicable), FDA, and Sponsor. The IRB and other applicable recipients will review study

progress on an annual basis.

**E. Study Report Outline for the Independent Monitors(s) (Interim or Annual Reports)**

Study Report tables will be generated only from aggregate (not by group assignment) baseline and aggregate safety data for the study population. A separate Closed Safety Report, with masked group baseline and safety data, will be generated for the Independent Monitor(s) by a designated unmasked member of the team but will not be reviewed by the study team.

## IV. Informed Consent

Written informed consent will be obtained from each subject at entry into the study. Informed consent is obtained by the following process:

1. The subject (If applicable, parent/guardian) will be asked to review the study consent form.
2. The PI or Co-I will meet with the subject to review the form, to confirm the subject's understanding of the study, and to answer any questions that the subject might have.
3. Once the subject demonstrates understanding of the study and agrees to participate in the study, the consent will be signed in the presence of the PI (or Co-I) and a witness.

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## 19. Appendices

### Appendix A: ECOG/Karnofsky Performance Status Criteria

| ECOG Performance Status Scale | | Karnofsky Performance Scale | |
|---|---|---|---|
| **Grade** | **Descriptions** | **Percent** | **Description** |
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. | 100 | Normal, no complaints, no evidence of disease. |
| | | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work). | 80 | Normal activity with effort; some signs or symptoms of disease. |
| | | 70 | Cares for self, unable to carry on normal activity or to do active work. |
| 2 | In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours. | 60 | Requires occasional assistance, but is able to care for most of his/her needs. |
| | | 50 | Requires considerable assistance and frequent medical care. |
| 3 | In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. | 40 | Disabled, requires special care and assistance |
| | | 30 | Severely disabled, hospitalization indicated. Death no imminent. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. | 20 | Very sick, hospitalization indicated. Death not imminent. |
| | | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead | 0 | Dead |

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## Appendix B: New York Heart Association Classifications

| Class | Description |
|---|---|
| I | Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain. |
| II | Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain. |
| III | Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain. |
| IV | Patients with cardiac disease resulting in inability to carry on physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present event at rest. If any physical activity is undertaken, discomfort is increased. |

This table is an excerpt from the Oxford Textbook of Medicine, 2nd ed. Oxford; New York: Oxford University Press, 1987, p. 2228.

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**Appendix C: Version 4.03 (dated June-14-2010)**

**CTCAE Files**

**NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 data files** and related documents are published here. The most current release files appear in this directory:

| Files: Booklet | Content |
|---|---|
| CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf | Most recent release of core terminology: PDF document, traditional small booklet format. |

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

**Appendix D:** Modified irRC, derived from RECIST 1.1

**Overall responses derived from changes in index, non-index, and new lesions**

| Measurable Response | Non-Measurable Response | | Overall Response Using Modified irRC |
|---|---|---|---|
| **Index and New, Measurable Lesions (Tumor Burden)** | **Non-Index Lesions** | **New, Non-Measurable Lesions** | |
| Decrease 100% | Absent | Absent | irCR* |
| Decrease 100% | Stable | Any | irPR* |
| Decrease 100% | Unequivocal progression | Any | irPR* |
| Decrease ≥ 30% | Absent / Stable | Any | irPR* |
| Decrease ≥ 30% | Unequivocal progression | Any | irPR* |
| Decrease < 30% to increase < 20% | Absent / Stable | Any | irSD |
| Decrease < 30% to increase < 20% | Unequivocal progression | Any | irSD |
| Increase ≥ 20% | Any | Any | irPD |

***** Assuming that the response (irCR and irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart (normally it should be done 6 weeks apart).

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Key Milestones:

FPFV / First Dose:

LPLV / Last Subject completed:

Database Lock:

Final Report:

Sponsoring Department: HMHCC &HMHRI

Prepared by: _____ Date _____